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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

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MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

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The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

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Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· Objective: Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

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· Results: Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· Conclusion: Directly supported by data, along with clinical implications.

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Below the abstract provide 3 to 5 keywords. Abbreviations should not be used as keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

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Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	2,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

Materials and Methods

Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report

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outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

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Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

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The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

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The main text of case reports should be structured with the following subheadings:

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Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2021;18:167-174

Serum sclerostin level and its relation to subclinical atherosclerosis in the polycystic ovary syndrome phenotypes: A prospective controlled study

Serum sclerostin seviyesinin polikistik over sendromu fenotiplerinde subklinik ateroskleroz ile ilişkisi: Prospektif kontrollü çalışma

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Abstract

Objective: We aim to study the relationship between atherosclerosis and serum sclerostin levels in different phenotypes of polycystic ovary syndrome (PCOS).

Materials and Methods: A total of 134 women with PCOS and 33 age-matched controls participated in this study. Women with PCOS were further divided into subgroups based on their PCOS phenotypes: phenotype A (n=35), phenotype B (n=33), phenotype C (n=31), and phenotype D (n=35). Metabolic parameters, hormonal parameters, carotid intima-media thickness (CIMT), and sclerostin levels were compared among the PCOS phenotypes.

Results: Statistically significant differences occurred among groups regarding follicle-stimulating hormone, luteinizing hormone, estradiol, total cholesterol, low-density lipoprotein, Ferriman-Gallwey score, total testosterone, and free androgen index. The mean CIMT was statistically higher in all PCOS phenotypes than in controls. In subgroup comparison, phenotypes A and B had a higher body mass index (BMI) adjusted CIMT than other phenotypes, respectively (p=0.005). Serum sclerostin levels were higher in PCOS patients than in controls. A concentration of ≥6.297 ng/mL showed a sensitivity of 56% and a specificity of 69.7% to predict PCOS. The BMI-adjusted sclerostin level was significantly higher in phenotype C (20.3±0.7 ng/mL) than in other phenotypes.

Conclusion: Patients with phenotypes A and B seem to have an increased risk for atherosclerosis. Although sclerostin was higher in PCOS patients, we could not demonstrate the relation between sclerostin and atherosclerosis in different PCOS phenotypes.

Keywords: Atherosclerosis, carotid intima-media thickness, polycystic ovary syndrome, sclerostin

Öz

Amaç: Bu çalışmada amacımız polikistik over sendromu (PKOS) fenotiplerinde serum sclerostin seviyelerini belirlemek ve ateroskleroz ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: Yüz otuz dört PKOS tanılı hasta ve 33 kontrol hastası çalışmaya dahil edildi. PKOS hastaları fenotiplerine göre 4 alt gruba ayrıldı; fenotip A (n=35), fenotip B (n=33), fenotip C (n=31) ve fenotip D (n=35). PKOS fenotiplerinde metabolik, hormonal değişkenler ile karotis intima media kalınlığı (KIMK) ve sclerostin seviyeleri karşılaştırıldı.

Bulgular: Gruplar arasında folikül stimülan hormon, luteinizan hormon, estradiol, total kolesterol, düşük yoğunluklu lipoprotein, Ferriman-Gallwey skoru, total testesteron ve serbest androjen indeksinde istatistiksel olarak farklılıklar tespit edildi. Ortalama KIMK değeri tüm PKOS fenotiplerinde kontrollere göre istatistiksel olarak yüksek bulundu. Alt grup analizinde sırasıyla fenotip A ve B hastalarında vücut kitle indeksi (VKİ) düzeltilmiş KIMK değeri diğer gruplara göre yüksek ölçüldü (p=0,005). Serum sclerostin seviyeleri PKOS hastalarında kontrollere göre yüksek tespit edildi. Sclerostinin 6,297 ng/mL ve üzeri

PRECIS: PCOS patients with phenotype A and B seem to have increased risk for atherosclerosis. Although sclerostin was found higher in PCOS patients than controls, a relation couldn't be demonstrated between sclerostin and atherosclerosis in PCOS patients.

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değerlerinde PKOS hastalığını %56 sensitivite ve %69,7 spesifite ile tahmin edebildiği gösterildi. VKİ düzeltilmiş sclerostin seviyeleri fenotip C hastalarında diğer fenotiplere kıyasla istatistiksel olarak yüksek bulundu (20,3±0,7 ng/mL).

Sonuç: Fenotip A ve B'ye sahip PKOS hastaları ateroskleroz için artmış riske sahip görünmektedirler. Sclerostin seviyeleri PKOS hastalarında genel olarak yüksek bulunsa da, PKOS fenotiplerinde serum sclerostin ile subklinik ateroskleroz ilişkisi net olarak tanımlanamamıştır.

Anahtar Kelimeler: Aterosklerozis, karotis intima media kalınlığı, sclerostin, polikistik over sendromu

Introduction

Polycystic ovary syndrome (PCOS) is recognized as one of the most common endocrine/metabolic disorders affecting women worldwide. Its prevalence was reported as 10%, according to the Rotterdam criteria[1]. These criteria define PCOS for those with at least two of the following three conditions: oligo-anovulation, clinical/biochemical hyperandrogenism, and polycystic ovary (PCO) appearance on ultrasonography[2]. According to three diagnostic Rotterdam criteria, PCOS is divided into four phenotypes because of the heterogeneity of the signs and symptoms. The spectrum extends from women showing the classical features to those who may not even have features of hyperandrogenism: phenotype A (hyperandrogenism + PCO + oligo-anovulation), phenotype B (hyperandrogenism + oligo-anovulation), phenotype C (hyperandrogenism + PCO), and phenotype D (oligo-anovulation + PCO)[3]. In a review, most PCOS patients (60%) were categorized into phenotype A[4]. Phenotype A is known as full, phenotype B is known as classical, phenotype C is known as ovulatory, and phenotype D is known as non-hyperandrogenic PCOS[3]. In women with full and classical phenotypes of PCOS (phenotypes A and B), higher levels of luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratios, higher risk of insulin resistance (IR) [5], and metabolic disturbances[6], and more severe forms of atherogenic dyslipidemia[7] existed than did ovulatory and nonhyperandrogenic PCOS phenotypes. Studies show the association between subclinical atherosclerosis, endothelial dysfunction, and PCOS[8,9]. Studying the clinical, hormonal, and metabolic parameters of these phenotypes can help to specify the riskiest phenotypes.

Sclerostin is a cysteine-rich glycoprotein, which is predominately secreted by osteocytes[10]. It retards the Wingless-type Mouse mammary tumor virus integration site (Wnt) pathway via binding its LRP5/6 coreceptors[10]. LRP5/6 acts to regulate glucose and lipid metabolism. Mutations in LRP5/6 are responsible for hypertension, type 2 diabetes, high low-density lipoprotein (LDL) cholesterol and triglycerides (TG), and early coronary artery disease[11]. Defective Wnt and LRP5/6 signaling could cause atherosclerosis[12]. Also, sclerostin is associated with subclinical atherosclerosis in type 2 diabetes mellitus and chronic kidney disease patients[13,14].

In the literature, there is no data regarding serum sclerostin levels in PCOS patients. We aim to determine whether there is an alteration in sclerostin levels and any link between sclerostin levels and subclinical atherosclerosis in women with PCOS.

Materials and Methods

Study Design and Patient Selection

This prospective case-control study was conducted from June 2019 to July 2020 at a tertiary hospital with approval from the local institutional review board (reg. no. 2019/184). All patients gave written informed consent after they were informed about the study protocol and aim. The study was conducted in accordance with the Helsinki Declaration Principles.

All the women presented with one or two or all of the following complaints: oligomenorrhea, hirsutism, and infertility, and were systematically evaluated in our outpatient clinic. During the systematic evaluation, all the participants underwent a physical examination and sonographic evaluation and were screened for hormonal abnormalities. A total of 134 women with PCOS and 33 age-matched controls participated in this study. The diagnosis of PCOS was made according to the Rotterdam criteria[2] in the presence of at least two of the following: oligomenorrhea and/or anovulation, biochemical and/or clinical hyperandrogenism, and ultrasound appearance of PCO. Other etiologies, such as congenital adrenal hyperplasia, virilizing tumor, Cushing syndrome, thyroid dysfunction, prolactinoma, diabetes, hypertension, and other cardiovascular diseases, were considered exclusion criteria. Patients who had taken any medication that could affect gonadotropin, lipid, and carbohydrate metabolism during the previous three months were excluded from the study. No subject smoked or consumed alcohol. Women with PCOS were further divided into subgroups based on the PCOS phenotypes: phenotype A, phenotype B, phenotype C, and phenotype D (as mentioned above). As controls, 33 age-matched women who had regular menses and had no clinical or biochemical hyperandrogenism or PCO were eligible. Oligomenorrhea was diagnosed in patients with cycles longer than 35-day intervals or with fewer than eight cycles of menstruation during the past 12 months, and amenorrhea was determined in the absence of menstruation for three consecutive months. Clinical hyperandrogenism was defined as the presence of hirsutism and/or acne and/or alopecia. Hirsutism was evaluated according to the modified Ferriman-Gallwey score, and patients with a total score ≥8 were considered hirsute[15]. Biochemical hyperandrogenism was defined as total testosterone greater than 80 ng/dL. PCO was defined as the presence of 12 or more ovarian cysts with 2-9 mm diameter per ovary and/or ovarian volume (OV) ≥10 cm^3[2].

The initial physical examination included weight, height, waist and hip circumferences to calculate the waist-hip ratio (WHR)

and BMI. Waist and hip circumferences were measured as described by Dilbaz et al.[16]. BMI was calculated as the ratio of weight divided by height squared (kg/m^2). The patients with a BMI value of ≥18 kg/m^2 and ≤30 kg/m^2 were enrolled in the study. The BMI, WHR, and hirsutism scores were assessed by the same physician (FNİÇ) for all subjects.

All regularly menstruating women were scanned by ultrasound on cycle days 3-5, whereas oligo/amenorrheic women were scanned between days 3 and 5 after progestin-induced withdrawal of bleeding. Ovarian morphology was evaluated with transvaginal ultrasound (Esaote My Lab Seven, 2018, Italy) by the same physician (FNİÇ). The size and the total number of antral ovarian follicles 2-9 mm (AFC) were evaluated. OV was measured using a simplified formula for the volume of a prolate ellipsoid: OV=0.5 × length × height × width[17].

Biochemical Analysis

All blood samples were taken in the early follicular phase (day 2-5 of the menstrual cycle) in the morning after an overnight fast. Then, serum samples were stored at -70 °C until further analysis. Routine chemistry laboratory investigations included fasting blood glucose, total cholesterol, high-density lipoprotein (HDL), LDL, and TG were measured using the Beckman Synchron AU 5800 (Beckman Coulter, USA), according to standard laboratory techniques. Routine hormonal analysis of LH, FSH, estradiol (E2), thyroid-stimulating hormone, fasting insulin, and total testosterone was done by chemiluminescence methods using ADVIA Centaur1 XP immunoassay system (Siemens Diagnostics, Germany). Sex hormone-binding globulin (SHBG) was measured with the YLBIONT SHBG ELISA Kit (Shanghai YL Biotech Co., Ltd) in accordance with the manufacturer's guidelines. SHBG was reported in nanomoles per liter. IR was determined by the homeostasis model assessment (HOMA-IR) index [fasting glucose (mg/dL) × fasting insulin (mU/mL)/405]. Free androgen index (FAI) was calculated according to the equation total testosterone (nmol/L) × 100/SHBG (nmol/L). Serum sclerostin was detected with the YLBIONT sclerostin ELISA Kit (Shanghai YL Biotech Co., Ltd) in accordance with the manufacturer's guidelines. Sclerostin measurements are reported in nanograms per milliliter.

Carotid Artery Studies

Carotid intima-media thickness (CIMT) was determined by the same radiologist (AA) using high-resolution ultrasound (Toshiba Aplio 500, Japan) with a multifrequency linear probe (5-12 MHz) and standardized image settings. The bilateral distal common carotid arteries, 1 cm proximal to the bifurcation, were imaged during end-diastole, with the patient in a supine position and the neck slightly extended. CIMT was defined as the distance between the leading edges of the lumen-intima interface and the media-adventitia interface of the far wall of the carotid artery; the mean of 6 recordings (3 on each side) was calculated. The highest value of the two sides was accepted for each patient. A CIMT higher than 0.9 mm was accepted as atherosclerosis[13,18].

Statistical Analysis

Data are presented as mean ± standard deviation, median (minimum-maximum), wherever appropriate, and analyzed using SPSS software (IBM statistics, version 21). Parameters of PCOS phenotypes and controls were compared using One-Way ANOVA or Kruskal-Wallis analysis. When the p-value from one-way ANOVA or Kruskal-Wallis test statistics was statistically significant for Post-hoc analysis, the Games-Howel or Mann-Whitney U test was applied. Analysis of covariance (ANCOVA) was used to determine significant differences in CIMT and sclerostin between cases and controls after adjusting for confounders. Age and BMI were used for adjustment. Spearman correlation was used for correlation analysis of variables with CIMT and sclerostin. A receiver operating characteristic (ROC) analysis was also used for cutoff value, specificity, sensitivity, and AUC detection. A $p<0.05$ was considered significant.

Results

The clinical characteristics and steroid hormone levels of the study population are presented in Table 1. Statistically significant differences were found among PCOS phenotypes and the control group regarding FSH, LH, estradiol, total cholesterol, LDL, Ferriman-Gallwey score, total testosterone, FAI, over volume, and antral follicle count. The BMI significantly differed between groups (p=0.028). FSH was significantly lower, and LH was significantly higher in phenotypes A and D than in controls, whereas E2 was significantly lower in phenotypes B and C than in the control group. Estradiol was higher in phenotype A than in phenotypes B and C in the subgroup analysis. All PCOS patients had higher total cholesterol, LDL, and TG levels than the control group. In the phenotype C group, compared with phenotypes A and B, LDL and total cholesterol levels were significantly lower. In clinical hyperandrogenism evaluation, control patients had statistically lower FGS than phenotypes A, B, and C. In the biochemical hyperandrogenism evaluation, the control group had significantly lower mean total testosterone levels than phenotypes A, B, and C. Phenotype D had lower total testosterone levels than other phenotypes statistically. Therefore, phenotype D was known as the nonhyperandrogenic group. AFC was statistically higher in all PCOS patients but not in controls, and the lowest AFC was found in phenotype B in the phenotype comparison. The mean CIMT was found statistically higher in phenotypes A and B than in controls.

Significant differences in CIMT persisted after adjusting for BMI. CIMT was higher in all PCOS phenotypes than in controls (p=0.015). In the subgroup analysis, phenotypes A and B had the highest mean CIMT (0.488±0.005 mm and 0.480±0.003 mm), whereas phenotype D had the lowest mean CIMT (0.460±0.005 mm). Sclerostin levels were detected significantly higher only in phenotype C than in controls. The BMI-adjusted

Table 1. Clinical, biochemical, and radiological parameters in the PCOS phenotypes and control group

Variables	Control (n=33)	Phenotype A (n=35)	Phenotype B (n=33)	Phenotype C (n=31)	Phenotype D (n=35)	p-value
Age (years)	22.2±3.5	22.4±3.9	20.8±2.2	21.5±2.2	21.9±2.9	0.192
BMI	22.9±3.9	23.9±3.5	22.3±2.2	21.8±3.4	23.9±3.4	**0.028***
WHR	0.8±0.07	0.8±0.07	0.8±0.06	0.8±0.04	0.8±0.07	0.059
FSH (mIU/mL)	8.4±3.0	6.2±1.3	6.8±2.1	6.9±2.0	6.6±1.6	**0.008**[a,d]
LH (mIU/mL)	5.4±3.0	7.8±3.8	7.3±3.3	6.6±5.3	9.0±6.5	**0.010**[a,d]
E2 (mIU/mL)	47.5±20.8	47.0±17.4	29.8±10.4	35.4±11.6	44.7±23.5	**<0.001**[b,c,e,f,i]
Prolactin (µg/L)	12.1±5.2	12.2±5.1	16.3±8.1	14.6±8.8	14.9±8.3	0.075
TSH (mIU /L)	2.6±1.4	2.0±1.4	1.7±0.7	2.0±1.0	2.0±1.0	0.051
Fasting glucose (mg/dL)	86.9±8.0	86.7±9.0	87.5±9.1	87.5±11.9	85.8±8.3	0.942
Insulin (µıu/mL)	8.4±7.3	12.2±9.1	11.5±6.6	12.1±9.4	9.7±6.5	0.202
HOMA-IR	1.8±1.6	2.7±2.3	2.5±1.4	2.8±2.4	2.1±1.5	0.187
Total cholesterol (mg/dL)	149.1±14.9	185.1±46.3	173.4±24.4	156.7±24.8	166.4±30.6	**<0.001**[a,b,d,f]
LDL (mg/dL)	78.4±12.1	109.2±37.5	100.9±15.6	83.8±23.2	96.5±26.0	**<0.001**[a,b,d,f,h,i]
HDL (mg/dL)	55.5±9.9	56.0±10.2	53.4±10.8	55.4±12.8	51.7±8.7	0.393
TG (mg/dL)	85.8±30.3	99.3±51.1	95.8±24.3	87.7±26.3	91.0±60.1	0.659
FGS	0 (0-6)	13.0 (8-28)	12.0 (8-29)	13.0 (9-19)	3.0 (3-6)	**<0.001**[a,b,c,g,i,j]
Total testosterone (ng/dL)	32.4±10.6	52.4±19.6	63.9±26.1	49.1±21.2	34.3±10.6	**<0.001**[a,b,c,g,i,j]
SHBG (nmol/L)	93.0±125.3	128.9±148.2	71.6±77.7	116.6±119.0	79.0±93.2	0.201
FAI	2.1±1.4	3.9±4.4	6.2±6.8	3.7±5.0	3.0±2.9	**0.005**[b]
Over volume	10.0±4.2	10.7±4.4	9.0±2.2	15.3±4.3	9.2±6.6	**<0.001**[c,f,h,j]
AFC	7.64±4.2	14.51±1.4	10.7±1.7	14.5±1.6	13.5±2.2	**<0.001**[a,b,c,d,e,h,i]

ANOVA (Welch), Kruskal-Wallis, and post-hoc analysis (Games-Howell and Mann-Whitney U test) were used. The bold values represent statistically significant (p<0.05).
*Statistical difference was not found in the binary comparison.
BMI: Body mass index, WHR: Waist-hip ratio, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid-stimulating hormone. HOMA-IR: homeostasis model assessment index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TG: Triglycerides, FGS: Ferriman-Gallwey score, SHBG: Sex hormone-binding globulin, FAI: Free androgen index, AFC: Antral follicle count, PCOS: Polycystic ovary syndrome
[a]: between control and phenotype A, [b]: between control and phenotype B, [c]: between control and phenotype C, [d]: between control and phenotype D, [e]: between phenotype A and phenotype B, [f]: between phenotype A and phenotype C, [g]: between phenotype A and phenotype D, [h]: between phenotype B and phenotype C, [i]: between phenotype B and phenotype D, [j]: between phenotype C and phenotype D

Table 2. CIMT and sclerostin in the PCOS phenotypes and control group

Variables	Phenotype A	Phenotype B	Phenotype C	Phenotype D	Control	p-value
CIMT (mm)						
Unadjusted	0.49±0.07	0.48±0.06	0.46±0.05	0.46±0.04	0.44±0.06	**0.015**[a,b]
Adjusted[1]	0.488±0.005	0.480±0.003	0.464±0.005	0.460±0.005	0.438±0.005	**0.005**[a,b,c,d,e,f,g,h,i,j]
Sclerostin (ng/mL)						
Unadjusted	13.7±15.1	13.4±13.1	20.3±18.4	17.2±0.2	8.5±11.5	**0.025**[c]
Adjusted[1]	13.7±0.7	13.4±0.4	20.3±0.7	17.2±0.7	8.5±0.8	**<0.001**[a,b,c,d,e,f,g,h,j]

ANOVA (Welch); Kruskal-Wallis and post-hoc analysis (Games-Howell and Mann-Whitney U test) were used. The bold values represent statistically significant (p<0.05).
CIMT: Carotid intima-media thickness, PCOS: Polycystic ovary syndrome
[1]Analysis of covariance (ANCOVA), adjusted for BMI, [a]: between control and phenotype A, [b]: between control and phenotype B, [c]: between control and phenotype C, [d]: between control and phenotype D, [e]: between phenotype A and phenotype B, [f]: between phenotype A and phenotype C, [g]: between phenotype A and phenotype D, [h]: between phenotype B and phenotype C, [i]: between phenotype B and phenotype D, [j]: between phenotype C and phenotype D

sclerostin level (20.3±0.7 ng/mL) was significantly higher in phenotype C, whereas phenotypes A and B had the lowest sclerostin values (Table 2). A weak positive correlation was found in the correlation of sclerostin, CIMT, and cardiovascular risk factors, such as BMI, WHR, total cholesterol, LDL, HDL, triglyceride, and HOMA-IR between sclerostin and total cholesterol A weak negative correlation was found between sclerostin and HDL (p=0.029 and p=0.016, respectively) (Table 3). We compared CIMT and sclerostin levels among PCOS patients with and without biochemical hyperandrogenism. Although the hyperandrogenic group had a higher mean CMIT (0.48±0.06 mm) and sclerostin levels (16.9±16.4 ng/mL), the two groups were statistically similar (p=0.421 and p=0.192, respectively) (Table 4).

A ROC curve analysis was performed to evaluate the usefulness of sclerostin as a marker for PCOS. It showed an area under the curve of (0.656, p=0.006) for predicting PCOS. A concentration of 6.297 ng/mL showed a sensitivity of 56% and a specificity of 69.7% to identify an increased risk for PCOS. The diagnostic performance of sclerostin revealed a specific marker rather than a sensitive marker (Table 5, Figure 1).

Table 3. The correlations of cardiovascular risk factors and CIMT and sclerostin in the PCOS phenotypes

	CIMT (mm)		Sclerostin (ng/mL)	
	r	p-value	r	p-value
BMI	0.052	0.504	0.084	0.337
WHR	0.069	0.377	0.147	0.090
Total cholesterol	0.013	0.866	0.169	**0.029**
LDL	0.004	0.963	0.139	0.072
HDL	-0.043	0.580	-0.186	**0.016**
Triglyceride	0.022	0.779	-0.063	0.418
HOMA-IR	0.016	0.841	-0.141	0.104
Sclerostin	0.079	0.310	-	.

CIMT: Carotid intima-media thickness, BMI: Body mass index, WHR: Waist-hip ratio, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HOMA-IR: Homeostasis model assessment index.
Spearman correlation was used. The bold values represent statistically significant (p<0.05)

Table 4. The comparison of CIMT and sclerostin in PCOS patients with and without hyperandrogenism

	Hyperandrogenic PCOS group n=99	Normoadrogenic PCOS group n=35	p-value
CIMT (mm)	0.48±0.06	0.46±0.04	0.421
Sclerostin (ng/mL)	16.9±16.4	13.7±15.1	0.192

CIMT: Carotid intima-media thickness
Student t-test was used

Table 5. Best cutoff value, specificity, sensitivity, and AUC of sclerostin

Cut-off	Cutoff	Specificity	Sensitivity	AUC (95% CI)	p-value
Sclerostin (ng/mL)	≥6.297	69.7	56.0	0.656 (0.556-0.755)	**0.006**

AUC: area under the curve, CI: confidence interval
The bold values represent statistically significant (p<0.05)

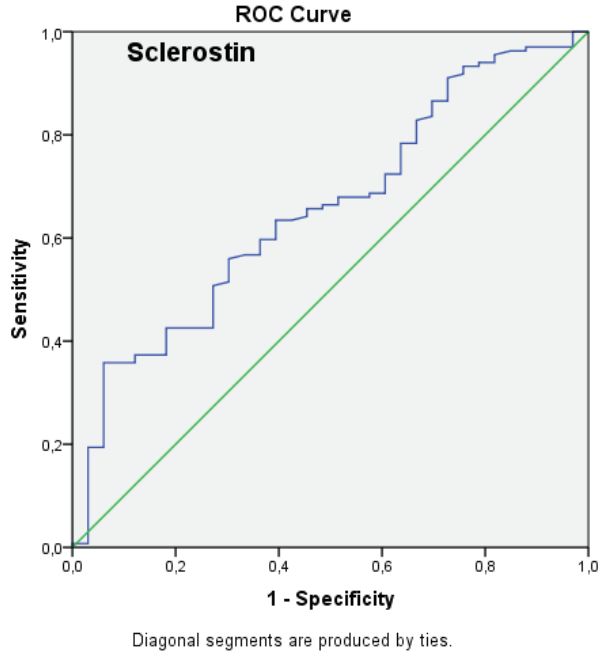

Figure 1. ROC curve for sclerostin (ng/mL) to the prediction of PCOS

ROC: Receiver operating characteristic, PCOS: Polycystic ovary syndrome

Discussion

This study demonstrated higher levels of sclerostin and CMIT in PCOS patients than those in healthy subjects. We also found that full and classic PCOS patients had the highest BMI-adjusted CMIT levels and ovulatory PCOS patients had the highest serum sclerostin levels among the phenotypes. The results point out that patients with phenotypes A and B might develop more atherosclerosis than other phenotypes.

PCOS was accepted as a major independent risk factor for atherosclerotic cardiovascular disease[19]. Progestins (especially those with androgenic activity), oral contraceptives, and obesity are secondary causes of dyslipidemia[19]. PCOS patients have an increased risk for atherosclerosis because of the disease's nature and its treatment. Women with hyperandrogenic PCOS (phenotype A, B, and C) had a higher prevalence of cardiovascular disease compared with the nonhyperandrogenic PCOS phenotype (phenotype D) even after adjusting for age,

smoking, BMI, and ethnic origin[20]. In a study by Rizzo et al.[21], which compared ovulatory PCOS (phenotype C) with other phenotypes, ovulatory women with PCOS had milder forms of atherogenic dyslipidemia than women with anovulatory PCOS. Atherosclerosis risk was evaluated in this study by CIMT measurements in different PCOS phenotypes. In a study of young and normal-weight PCOS patients[22], mean CIMT was found significantly higher than that in controls (0.746±0.106, 0.608±0.105, respectively). Yildir et al.[23] reported the mean CIMT as 0.50±0.11 in overweight PCOS patients, but the difference was not significant. In a study that evaluated cardiovascular disease risk by CIMT measurements in different PCOS phenotypes, ovulatory PCOS patients (phenotype C) had lower CIMT values than the other three phenotypes[16]. The authors attributed this to anovulation as having a major negative effect on cardiovascular risk. Unlike the previous studies in PCOS patients evaluating CIMT, we also analyzed CIMT values adjusted for BMI. Significant differences occurred in CIMT among phenotypes after adjusting for BMI, and we found the CIMT values were highest to the lowest as phenotypes A, B, C, and D, respectively. In all the phenotypes in our study group, the means of CIMT were lower than the atherosclerosis cutoff. Because of the young age of the patients, the metabolic dysfunction might require additional time to become clinically evident. Different mean CIMT values for PCOS patients than in previous studies might be attributed to the BMI-adjusted analysis in this study. Atherosclerosis risk was significantly associated with high LDL, total cholesterol levels, and BMI[16]. Our phenotype A and B patients had higher lipid parameters and BMI than the other phenotypes. These data imply that anovulatory patients with hyperandrogenism had more atherosclerosis and cardiovascular risk than others. The prevalence of metabolic abnormalities was lower in phenotype D[20], so it might suggest that androgens play a significant role in increasing the risk of metabolic and cardiovascular diseases.

Recent data suggest Wnt signaling occurs in early atherogenesis[24]. Sclerostin, a soluble inhibitor of the Wnt signaling pathway, was reported to have a close relation to atherosclerosis in patients with chronic diseases[13,25]. We researched the relation between PCOS and sclerostin first in the literature. We found that BMI-adjusted serum sclerostin levels were significantly higher in women with PCOS than in healthy women. Sclerostin levels higher than 6.297 ng/mL could predict PCOS with a 56% sensitivity. In the phenotype comparison, ovulatory PCOS patients (phenotype C) had the highest BMI-adjusted serum sclerostin levels following non-hyperandrogenic PCOS patients (phenotype D), and the lowest sclerostin levels were in the classic PCOS patients (phenotype B). We could not find a correlation between sclerostin and CIMT levels. This result could be related to CIMT values significantly higher in our PCOS patients than controls, although they were not as high as the atherosclerosis cut-off (0.9 mm). If we had an older and overweight/obese study population, atherosclerosis could be more significant in patients to show more clearly the relation between sclerostin and PCOS patients.

Atherosclerosis risk was significantly associated with the lipid profile and BMI. In the current study, sclerostin showed a weak positive correlation with total cholesterol and a weak negative correlation with HDL. Excess androgen appears to regulate metabolic and cardiovascular changes as an independent parameter[16]. While we have compared the parameters between PCOS patients with and without biochemical hyperandrogenism, we found the mean CIMT and sclerostin levels higher in the hyperandrogenic group. However, the two groups were found to be statistically similar.

We found significantly lower FSH and higher LH levels in patients with full and non-hyperandrogenic PCOS than in the control group, but the difference was not statistically significant between phenotypes. In the lipid parameters' comparison, the phenotypes A and B had the highest total cholesterol and LDL levels in PCOS patients. In contrast, the ovulatory PCOS (phenotype C) group had the lowest total cholesterol and LDL levels. In the literature, controversial data exist about the lipid profile of PCOS phenotypes. Daan et al.[20] showed that in hyperandrogenic PCOS women, lipid profiles were significantly more disturbed, and metabolic syndrome was more prevalent than non-hyperandrogenic PCOS women. In contrast, some studies reported no difference existed in the cholesterol profile between PCOS phenotypes[3]. Dilbaz et al.[16] reported that phenotype C had the lowest mean LDL, total cholesterol levels, and BMI was similar to our results. Dyslipidemia is generally related to hyperandrogenism and obesity. Thus, phenotypes A and B had higher BMI levels and higher total testosterone levels. Phenotype C had lower BMI levels and lower total testosterone levels than other phenotypes had, and this could have contributed to the lipid profile.

PCOS is a metabolic disorder associated with IR. Insulin and HOMA-IR levels were higher in PCOS patients than in controls, but IR was not statistically significant among groups. Ozay et al.[26] reported that HOMA-IR values had the same distribution among the four PCOS phenotypes with similar BMI values to ours. IR was compared in overweight PCOS patients with different phenotypes and was higher in phenotypes B and D than in other phenotypes[27]. Our results might be because our study was conducted on normal-weight PCOS patients.

Study Limitations

Our study has some strengths and limitations. To the best of our knowledge, this is the first published study that investigates the relationship between sclerostin and atherosclerosis in PCOS patients. PCOS consists of a broad spectrum of patients with heterogenic characteristics. This study tried to present the heterogeneity of phenotypes and demonstrate the atherosclerosis risk among groups. Atherosclerosis was evaluated using radiological and biochemical techniques. The

limitations of this study include the young age and low BMI of the participants, the small sample size, and the lack of enough data on individual atherosclerosis risk factors.

Conclusion

The different PCOS phenotypes present similarities in the same group and vary regarding endocrine and metabolic parameters. It is essential to identify the risk groups to design a screening and follow-up program for them. We conclude that atherosclerosis risk was higher in PCOS patients with phenotypes A and B. Screening these patients closely may alter and modify the risk factors for future cardiovascular disease. Although sclerostin was higher in PCOS patients, more studies are required to define the association of sclerostin and atherosclerosis in different PCOS phenotypes.

Ethics

Ethics Committee Approval: This prospective case-control study was conducted from June 2019 to July 2020 at a tertiary hospital with approval from the local institutional review board (reg. no. 2019/184).
Informed Consent: All patients gave written informed consent after they were informed about the study protocol and aim.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: F.N.İ.Ç., Design: F.N.İ.Ç., Data Collection or Processing: A.A., A.K., Analysis or Interpretation: Ü.C., E.Ç., Literature Search: O.G., Writing: F.N.İ.Ç.
Conflict of Interest: No conflict of interest was declared by the authors.
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The role of adropin, salusin-α, netrin-1, and nesfatin-1 in endometriosis and their association with insulin resistance

Endometriozisde adropin, salusin-α, netrin-1 ve nesfatin-1'in rolü ve bunların insülin direnci ile ilişkisi

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Abstract

Objective: The pathogenesis of endometriosis has not been clearly explained. Inflammatory factors of ectopic implantation and the growth of ectopic endometrial cells have been subjects of major interest. The number of studies evaluating salusin-α and nesfatin-1 markers in patients with endometriosis is limited. No studies have evaluated the levels of anti-inflammatory markers for adropin and netrin-1 in patients with endometriosis. This study investigates how some important inflammatory regulatory markers in the inflammatory process affect the pathogenesis of endometriosis and determines whether any relationship exists between serum levels of these parameters and endometriosis and insulin resistance.

Materials and Methods: This prospective study included 73 patients with endometriosis diagnosed histopathologically after laparoscopic surgery and 75 healthy controls. Serum adropin, salusin-α, netrin-1, and nesfatin-1 levels and homeostatic model assessment of insulin resistance (HOMA-IR) values of the participants were measured.

Results: The endometriosis group had significantly lower nesfatin-1 levels than the control group (3.0±0.53 vs 9.5±0.94, p=0.005). Between the patient and control groups, there was no difference regarding serum adropin, salusin-α, and netrin-1 levels (p=0.36, p=0.34, p=0.75, respectively). Nesfatin-1 had a significant positive correlation with adropin, salusin-α, and netrin-1 (r=0.563, p<0.01; r=0.738, p<0.01; r=0.700, p<0.01, respectively), but had a negative correlation with fasting blood glucose (r=–0.343, p<0.05). HOMA-IR values were comparable between both groups.

Conclusion: The lower nesfatin-1 levels leading to increased inflammatory pathway activity in patients with endometriosis might play a role in endometriosis pathogenesis. Without causing systemic insulin resistance, decreased nesfatin-1 might contribute to endometriosis pathogenesis locally by leading to the reduced insulin susceptibility of endometriosis cells.

Keywords: Endometriosis, adropin, salusin-α, netrin-1, nesfatin-1, insulin resistance

Öz

Amaç: Endometriozisin patogenezi henüz net bir şekilde aydınlatılamamıştır. Ektopik implantasyon ve endometriyal hücrelerin ektopik büyümesine ilişkin enflamatuvar faktörler büyük ilgi konusu olmuştur. Endometriozisli hastalarda salusin-α ve nesfatin-1 belirteçlerini değerlendiren çalışma sayısı sınırlıdır. Endometriozisli hastalarda adropin ve netrin-1 düzeylerini inceleyen bir çalışma bulunmamaktadır. Bu çalışmada, enflamatuvar süreçte rol oynayan bazı önemli enflamasyon düzenleyici belirteçlerin endometriozis patogenezine etkisini araştırmayı ve bu parametrelerin serum düzeyleri ile endometriozis ve insülin direnci arasında bir ilişki olup olmadığını ortaya çıkarmayı amaçladık.

Gereç ve Yöntemler: Bu prospektif çalışmada laparoskopik cerrahi sonrası histopatolojik olarak endometriozis tanısı alan 73 hasta ve kontrol grubu olarak 75 sağlıklı kadın çalışmaya dahil edildi. Katılımcıların serum adropin, salusin-α, netrin-1 ve nesfatin-1 düzeyleri ve insülin direncinin homeostatik model değerlendirmesi (HOMA-IR) değerleri ölçüldü.

Bulgular: Endometriozis grubunda nesfatin-1 düzeyleri kontrol grubuna göre anlamlı olarak düşüktü (3,0±0,53'e karşı 9,5±0,94, p=0,005). Her iki grup arasında serum adropin, salusin-α ve netrin-1 düzeyleri açısından anlamlı fark saptanmadı (p=0,36, p=0,34, p=0,75, sırasıyla). Nesfatin-1, adropin, salusin-α ve netrin-1 ile anlamlı pozitif korelasyon içindeyken (sırasıyla r=0,563, p<0,01; r=0,738, p<0,01; r=0,700, p<0,01), serum açlık kan şekeri düzeyi ile negatif bir korelasyonu vardı (r=-0,343, p<0,05). HOMA-IR değerleri her iki grup arasında benzerdi.

PRECIS: Without causing systemic insulin resistance, decreased nesfatin-1 might be contributing to the endometriosis pathogenesis locally by leading to a reduced insulin susceptibility of endometriomal cells.

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Sonuç: Endometriozis hastalarında enflamatuvar aktivitenin artmasına neden olan düşük nesfatin-1 düzeyi endometriozis patogenezinde rol oynayabilir. Sistemik insülin direncine neden olmadan, azalmış nesfatin-1 düzeyi, endometriozis hücrelerinin azalmış insülin duyarlılığına yol açarak lokal olarak endometriozis patogenezine katkıda bulunuyor olabilir.
Anahtar Kelimeler: Endometriozis, adropin, salusin-α, netrin-1, nesfatin-1, insülin resistansı

Introduction

Endometriosis is defined as the presence of endometrial glands and stromal cells outside the endometrial cavity. It affects from 7% to 10% of reproductive-age women[1]. The pathogenesis of endometriosis has not yet been clearly explained. However, recent papers suggest that inflammation plays an underlying role[2,3].

Studies demonstrated that circulatory adropin levels had a negative correlation with various inflammatory markers[4]. According to a study investigating the effects of adropin on glucose metabolism, adropin promoted carbohydrate oxidation, especially in skeletal muscle[5]. Another study found that adropin increased glucose tolerance, improved insulin resistance, and promoted carbohydrates over fat for fuel[6].

Salusin-α is a soluble peptide hormone found in various human tissues and plasma and acts in an endocrine and/or paracrine fashion. Salusin-α has angiogenic and anti-atherosclerotic effects[7]. Serum salusin-α levels are remarkably lower in confirmed coronary artery disease patients[8]. Salusin-α suppresses gene expression and protein levels of specific pro-inflammatory cytokines IL-6,IL-8, and IL-18 and, thus, attenuates inflammation in vascular endothelial cells[9].

A recent study asserts the anti-inflammatory influence of netrin-1 on endothelial cells[10]. In a study on newly diagnosed type 2 diabetes mellitus (DM) patients, netrin-1 was negatively correlated with the homeostatic model assessment of insulin resistance (HOMA-IR) and fasting blood glucose (FBG)[11].

Nesfatin-1 is secreted by the hypothalamic nuclei, which are responsible for controlling appetite[12]. An inverse correlation of serum nesfatin-1 levels with high-sensitivity C-reactive protein and the neutrophil percentage was reported[13]. Also, nesfatin-1 can inhibit the signaling pathway associated with inflammation by decreasing human recombinant high mobility group box 1 *(HMGB1)* gene expression, reducing inflammation, and oxidative stress in epithelial cells, thereby alleviating acute organ damage[14].Angiogenesis is a critical mechanism that allows the establishment and growth of endometriotic lesions. Several cytokines can either stimulate or inhibit the process of angiogenesis[15]. Inflammation stimulates the proliferation of quiescent vascular smooth muscle cells and fibroblasts[7]. A limited number of studies have evaluated salusin-α and nesfatin-1 markers-which play important roles in inflammatory pathways-in patients with endometriosis. No studies have evaluated the levels of anti-inflammatory markers for adropin and netrin-1 in patients with endometriosis. Similarly, the inflammatory process plays a role in the pathogenesis of insulin resistance. In addition, no research study has investigated the relationship between endometriosis and insulin resistance. This study investigates how some important inflammatory regulatory markers in the inflammatory process affect the pathogenesis of endometriosis and determines whether any relationship exists between serum levels of these parameters and endometriosis and insulin resistance.

Materials and Methods

Patient Selection

This prospective study was conducted in the infertility department of the Muğla Sıtkı Koçman University Faculty of Medicine between September 2019 and January 2021. The study included 73 patients with endometriosis diagnosed histopathologically after laparoscopic surgery and 75 healthy controls without endometriosis. Our study was approved by the Local Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (protocol ID: 22/08/2019-05). It was registered at Clinical Trials.gov (ID: NCT04371133). The study was conducted in accordance with the provisions of the Declaration of Helsinki. Informed consent forms were signed by all patients who participated in the study. Any patients with DM (2), chronic kidney disease (1), coronary artery disease (1), cerebrovascular accident (1), malignancy (0), rheumatoid disorder (2), liver disease (1), or active infections (3) were excluded from the study (Figure 1).

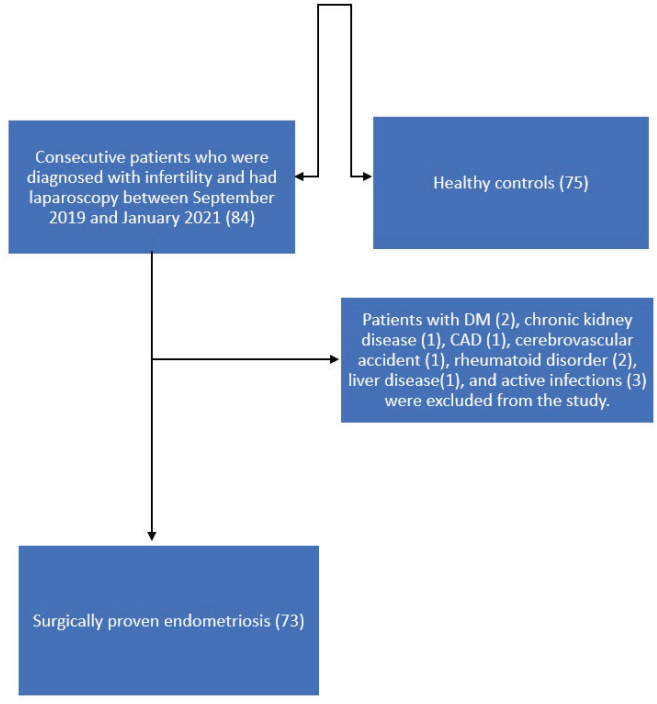

Figure 1. Flowchart for selecting the study population

Anthropometric Measurements

Body mass index (BMI) was calculated using the following formula: BMI: weight/(height)^2 (kg/m^2). Waist circumference measurements, in cm, were taken in parallel along the midpoint in between the lower edge of the 12th rib and the greater ischiadic (sciatic) notch. Hip circumference was measured at the maximum measurement of the buttocks in cm. The waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference.

Biochemical Analyses

After approximately eight hours of fasting, 4 cc of venous blood was drawn into a biochemistry tube from each patient during the preoperative period. Once collected, samples were left at room temperature for 30 min. The samples were then centrifuged for 5 min at 4000 rpm. Then, the serum were allocated into Eppendorf tubes and stored at -80 °C until the time of assay. When a sufficient number of patients and controls were reached, the Eppendorf tubes were taken out and thawed at room temperature to test for serum adropin, salusin-α, netrin-1, and nesfatin-1 levels. Thawed samples were measured on a Molecular Devices SpectraMax i3 Multi-Mode, Microplate Reader (batch number: SER 35 370-1448, Molecular Devices, LLC. made in Austria) using enzyme-linked immunosorbent assay (ELISA). Manufacturer instructions were followed to perform the tests. Serum adropin levels were measured using an adropin ELISA kit with article number: YLA0019HU; serum salusin-α levels were measured using a salusin-α ELISA kit with article number: YLA1761HU; serum netrin-1 levels were measured using a netrin-1 ELISA kit with article number: YLA1764HU; and finally, serum nesfatin-1 levels were measured using a nesfatin-1 ELISA kit with article number: YLA0715HU-all of which were commercially available under brand YL Biont. Measurements were recorded in ng/L for serum adropin levels, in pg/mL for salusin-α and netrin-1 levels, and in ng/mL for nesfatin-1 levels. Kits were stored at -20 °C until the time of use. Thyroid function and antibody testing were performed with a Cobas® c 8000 e602-3 series device (Roche, Switzerland) using the electrochemiluminescence method. Serum glucose and lipid values were determined spectrophotometrically using a Cobas® c 8000 c702 series device (Roche, Switzerland).

HOMA-IR

The HOMA-IR was determined using the following formula:
HOMA-IR: Fasting insulin (mU/L) × Fasting plasma glucose (mg/dL)/405.
Patients with a HOMA-IR value of 2.7 and above were considered to have insulin resistance[16].

Statistical Analysis

The authors determined the sample size for this study based on a preliminary evaluation[17]. From the differences, a two-tailed α value of 0.05 and a β value of 0.50 (study power: 95%), they ruled that at least 45 women in each group would be mandatory for an analysis comparing the two groups (G-Power 3 power analysis program). Therefore, assuming likely dropouts, it was determined that a minimum of 73 women should be included in each group.
The data were analyzed using the Statistical Package for Social Science (SPSS) 20.0 for Windows (SPSS Inc, Chicago, Illinois, USA). The distribution of the continuous variables was investigated using the Kolmogorov-Smirnov test. The significance of differences between the groups was determined using the Mann-Whitney U test (for non-normally distributed data) and the independent sample t-test (for normally distributed data). For the statistical evaluation of the categorical data, the chi-square test was used. The correlation of nesfatin-1 with adropin, salusin-α, netrin-1, and FBG was performed using Spearman's correlation analysis. Logistic regression analysis was performed. Statistical significance levels of the obtained data were interpreted using p-values. A p-value of <0.05 was considered statistically significant.

Results

A comparison of the clinical features of the patient and control group is provided in Table 1. On average, the group with endometriosis was significantly older than the control group (38.8±6.2 vs 35.0±5.2 years, p=0.02). Infertility and dyspareunia were more common in the endometriosis group than in the control group (36.4% vs 9.1%, p=0.03; 57.1% vs 20%, p=0.01, respectively).

Table 1. Clinical characteristics of endometriosis and control groups

Variables	Endometriosis (n=73)	Control (n=75)	p
Age (years)	38.8±6.2	35.0±5.2	0.02*
Gravidy (n)	1.6±0.4	1.6±0.5	0.97*
Parity (n)	1.3±0.5	1.2±0.4	0.68*
Abortus (n)	0.7±0.1	0.6±0.1	0.84*
Infertility, n (%)	8 (34.8)	2 (8)	0.03¥
Dysmenorrhea, n (%)	18 (78.3)	16 (64)	0.35¥
Dyspareunia, n (%)	12 (57.0)	5 (20)	0.01¥
Pelvic pain, n (%)	12 (52.2)	8 (32)	0.24¥
Menorrhagia, n (%)	7 (30.4)	3 (12)	0.12¥
GDM, n (%)	2 (11.8)	0	0.13¥
Gestational HT (%)	0	0	NS¥
Preeclampsia (%)	0	0	NS¥
BMI (kg/m^2)	25.0±4.7	24.9±4.0	0.27*
Waist-to-hip ratio	0.86±0.05	0.82±0.11	0.14*

GDM: Gestational diabetes mellitus, BMI: Body mass index, HT: Hypertension
*Independent samples t-test. ¥ Chi-square test

A comparison of the biochemical results of the patient and control group is shown in Table 2. In the endometriosis group, FBG was significantly higher (90.4±8.1 vs 82.1±6.7, p<0.01), although HDL-C and nesfatin-1 levels were significantly lower (52.6±9.0 vs 62.1±11.8, p=0.02; 3.0±0.53 vs 9.5±0.94, p=0.005, respectively). The patient and control groups did not differ regarding serum adropin, salusin-α, and netrin-1 levels (p=0.36, p=0.34, p=0.75, respectively).

Nesfatin-1 was positively correlated with adropin, salusin-α, and netrin-1, whereas it was negatively correlated with FBG (Table 3). No significant correlation between nesfatin-1 and any other parameters was detected (data not shown).

The multivariate analysis revealed that nesfatin-1 levels were associated with endometriosis. Individuals with decreased levels of nesfatin-1 had a 1.209-fold greater chance of exhibiting endometriosis (Table 4).

Table 2. Biochemical results of endometriosis and control groups

Variables	Endometriosis (n=73)	Control (n=75)	p
FBG (mg/dL)	90.4±8.1	82.1±6.7	**<0.01***
Insulin (μIU/mL)	1.8±0.32	1.4±0.28	0.53*
HOMA-IR	2.01±0.4	2.00±0.31	0.98*
Triglyceride (mg/dL)	131.7±24.3	109.3±19.1	0.25*
LDL-C (mg/dL)	112.2±21.7	98.6±18.9	0.13*
VLDL-C (mg/dL)	26.3±4.9	21.9±3.6	0.26*
HDL-C (mg/dL)	52.6±9.0	62.1±11.8	**0.02***
CRP	3.04±0.4	1.8±0.3	0.20*
Adropin (ng/L)	93.6±17.6	107.5±19.6	0.36**
Salusin-α (pg/mL)	415.9±83.1	511.8±92.2	0.34**
Netrin-1 (pg/mL)	441.8±74.2	472.2±84.0	0.75**
Nesfatin-1 (ng/mL)	3.0±0.53	9.5±0.94	**0.005****

FBG: Fasting blood glucose, HOMA-IR: Homeostatic model of assessment insulin resistance, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, CRP: C-reactive protein *Independent samples t-test. **Mann-Whitney U test

Discussion

In our cohort of patients with endometriosis, nesfatin-1 levels were decreased, but they positively correlated with adropin, salusin-α, and netrin-1 levels. In addition, we identified a negative correlation of nesfatin-1 with FBG and comparable levels of HOMA-IR in patients with endometriosis. Our results corroborate the hypothesis that inflammatory pathways may play a role in the pathogenesis of endometriosis.

Activation of nuclear factor kappa-B (NF-κB) contributes to the pathogenesis of endometriosis by stimulating inflammation and proliferation and inhibiting apoptosis in endometriosis cells[18]. A rat study has implied an anti-inflammatory and anti-apoptotic effect of nesfatin-1 after brain injury by inhibiting an NF-κB-related inflammatory response[19]. The above-mentioned data suggest that the lower nesfatin-1 levels detected in patients with endometriosis might be involved in the pathogenesis of endometriosis, causing enhanced activity of NF-κB-dependent inflammatory pathways. A prior study measured significantly lower nesfatin-1 levels in patients with endometriosis than controls, regardless of disease stage[20]. Our findings are consistent with the results of this study. This consistency suggests that decreased nesfatin-1 levels in patients with endometriosis may be related to decreased anti-inflammatory and anti-apoptotic effects of nesfatin-1, contributing to the etiopathogenesis of endometriosis.

Adropin decreases the mRNA expression of pro-inflammatory cytokines[21]. Our literature search revealed no studies that

Table 3. Correlation analysis for Nesfatin-1

Variables	Nesfatin-1 (ng/mL)	
	r	p
Adropin (ng/L)	0.563	**<0.01**
Salusin-α (pg/mL)	0.738	**<0.01**
Netrin-1 (pg/mL)	0.700	**<0.01**
FBG	-0.343	**<0.05**
HOMA-IR	0.117	**>0.05**

FBG: Fasting blood glucose, HOMA-IR: Homeostatic model of assessment-insulin resistance

Table 4. Logistic regression analysis

Variables	B	S.E.	Wald	df	Sig.	Exp (B)	95% CI for EXP (B)	
							Lower	Upper
Adropin	0.005	0.009	0.273	1	0.601	1.005	0.987	1.023
Salusin-α	-0.002	0.002	0.904	1	0.342	0.998	0.995	1.002
Netrin-1	0.000	0.002	0.031	1	0.860	1.000	0.997	1.003
Nesfatin-1	0.190	0.084	5.162	1	0.023	1.209	1.026	1.424
Constant	-0.517	0.741	0.487	1	0.485	0.596		

CI: Confidence interval

explore serum adropin levels in patients with endometriosis. On the other hand, there was no intergroup difference in our study. The disturbance of physiological angiogenesis mechanisms plays a role in the pathogenesis of some diseases in blood vessel over-proliferation, including endometriosis[22]. Increased plasma levels of salusin-α can promote the pro-angiogenic activity of some endothelial cells[17]. These findings suggest that salusin-α may play an essential role in inducing the development and progression of endometriosis. There were no significant correlations between plasma salusin-α levels with age, size of endometriotic cysts, bilaterality, or endometriotic focal number[17]. Our study also measured the salusin-α levels pre-operatively, which tended to be lower in the endometriosis group, and had a significant positive correlation with nesfatin-1 levels.

Netrin-1 results in an anti-inflammatory effect by inhibiting TNF-α-induced NF-κB activation and suppresses TNF-α-induced production of inflammatory cytokines[12]. Our literature search did not reveal any studies assessing serum netrin-1 levels of patients with endometriosis. There was no difference regarding serum netrin-1 levels between the two groups in our study. However, nesfatin-1 was positively correlated with netrin-1. A further and more extensive study that also measures adropin and netrin-1 levels of the follicular fluid might provide useful information about this topic.

Existing studies have identified favorable effects of nesfatin-1 on glucose metabolism that occurred with increased sensitivity to insulin in the brain[23]. In another study, patients with type 2 DM had lower nesfatin-1 levels than the control group, but no significant correlation was determined between nesftain-1 and HOMA-IR[24]. Similar to the existing data, nesfatin-1 was negatively correlated with FBG and comparable levels of HOMA-IR in the endometriosis and the control groups. However, we did not find a significant correlation of nesfatin-1 with HOMA-IR, which is a marker of systemic insulin resistance. To the best of our knowledge, insulin resistance of patients with endometriosis has not been previously investigated. The currently available data indicate increased glycolytic pathways in endometriosis cells, followed by elevated levels of lactate in follicular fluid. Elevated lactate levels, in turn, induce inflammation, angiogenesis, and cell proliferation[25,26]. Reduced nesfatin-1 levels may play a local role in endometriosis cells causing impaired insulin sensitivity and increased glycolytic pathways.

Study Limitations

The limitations of our study include the relatively small number of patients were enrolled, and only serum measurements of the molecules (adropin, salusin-α, netrin-1, and nesfatin-1) were performed.

Conclusion

Our study is the first of its kind to investigate adropin and netrin-1 levels in patients with endometriosis. Decreased nesfatin-1 levels and a positive correlation of nesfatin-1 with adropin, salusin-α, and netrin-1 in patients with endometriosis may have a combined effect on the inflammatory pathways that are believed to act in the multifactorial pathogenesis of endometriosis. More studies with larger sample sizes need to be performed to determine the levels of adropin, salusin-α, nesfatin-1, and netrin-1 in follicular fluid. Their roles in the pathogenesis of endometriosis can be clarified.

Ethics

Ethics Committee Approval: Our study was approved by the Local Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (protocol ID: 22/08/2019-05).

Informed Consent: Informed consent forms were signed by all patients who participated in the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.A., Design: E.A., Data Collection or Processing: B.S., Analysis or Interpretation: T.E., Literature Search: E.A., Writing: E.A., T.E.

Conflict of Interest:

Financial Disclosure: The authors declared that this study received no financial support.

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The combined effect of anti-D and non-D Rh antibodies in maternal alloimmunization

Maternal alloimmünizasyonda anti-D ve D dışı Rh antikorlarının kombine etkisi

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Abstract

Objective: This study aims to investigate the distribution of antibodies that cause hemolytic disease of the fetus and newborn (HDFN) and compare the clinical outcomes of pregnancies affected by anti-D and anti-D combined with non-D Rh alloimmunization.

Materials and Methods: We retrospectively searched and obtained the perinatal and neonatal data of patients with anti-D antibodies and anti-D combined with non-D Rh antibodies (anti-c, -C, -e, -E, and -Kell) from October 2015 to December 2018 at the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospital. Univariate and multiple logistic regression analyses and adjusted odds ratios with their confidence intervals were used to define independent risk factors for non-D antibody positive.

Results: The severe fetal hydrops rate was significantly higher in the anti-D combined non-D group (3/25, 12%) than in the anti-D group (1/128, 0.08%, p<0.001). The intrauterine transfusion (IUT) requirement in the anti-D combined non-D group (16/25, 64%) tended to be significantly higher than that in the anti-D group (5/128, 7.46%, p<0.001). The incidence of neonatal exchange transfusion, top-up transfusion, and postnatal phototherapy frequency in the anti-D combined non-D group was significantly higher than in the anti-D group.

Conclusion: Anti-D combined with another non-D Rh alloantibody resulted in significantly higher HDFN rates than the anti-D alloimmunized pregnancies. Also, anti-D in association with non-D Rh antibodies resulted in more severe HDFN requiring more invasive treatment procedures, including IUT, neonatal exchange transfusion, or top-up transfusion.

Keywords: Fetal anemia, hemolytic disease of the fetus and newborn, non-D antibodies, Rh alloimmunization

Öz

Amaç: Bu çalışma, fetüs ve yenidoğanın hemolitik hastalığına (FYHH) neden olan antikorların dağılımını araştırmayı ve anti-D ile birlikte D dışı Rh antikorlardan etkilenen gebeliklerin klinik sonuçlarını anti-D tarafından etkilenen gebeliklerle karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Sağlık Bilimleri Üniversitesi Kanuni Sultan Süleyman Eğitim ve Araştırma Hastanesi'nde Ekim 2015 - Aralık 2018 tarihleri arasında anti-D antikorları ve anti-D ile kombine D dışı (anti-c, -C, -e, -E, ve -Kell) Rh antikorları olan hastaların perinatal ve neonatal sonuçlarını geriye dönük olarak araştırdık. D dışı antikor pozitifliğinin varlığı için bağımsız risk faktörlerini tanımlamak için, tek değişkenli ve çoklu lojistik regresyon analizleri ve bunların güven aralıklarıyla ayarlanmış olasılık oranlarını kullandık.

Bulgular: Şiddetli fetal hidrops oranı anti-D ile kombine D dışı grupta (3/25, %12) anti-D grubundan (1/128, %0,08) anlamlı olarak daha yüksekti (p<0,001). Anti-D ile kombine D dışı gruptaki (16/25, %64) intrauterine transfüzyon (İUT) gereksinimi anti-D grubundan (5/128, %7,46) önemli ölçüde daha yüksekti (p<0,001). Anti-D ile kombine D dışı grupta neonatal kan değişimi, tamamlayıcı transfüzyon ve postnatal fototerapi sıklığı anti-D grubuna göre anlamlı olarak daha yüksekti.

Sonuç: Anti-D ile kombine D-dışı Rh alloantikoru ile oluşan gebelikler anti-D alloimmünize gebeliklerden önemli ölçüde daha yüksek FYHH oranları ile sonuçlanmıştır. Ayrıca, D-dışı Rh antikorları ile birlikte anti-D antikor varlığı, İUT, neonatal kan değişimi ve tamamlayıcı transfüzyon da dahil olmak üzere invaziv prosedürleri gerektiren daha ciddi FYHH ile sonuçlandı.

Anahtar Kelimeler: Fetal anemi, fetüs ve yenidoğanın hemolitik hastalığı, D dışı antikorlar, Rh alloimmünizasyonu

PRECIS: Anti-D combined with non-D Rh antibodies significantly influence the severity of fetal anemia compared with anti-D alone.

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Introduction

Red blood cell (RBC) alloimmunization occurs if an Rh-negative pregnant woman is exposed to Rh-positive fetal blood cells. This exposure leads to Rh-antibody development during pregnancy or delivery. RBC alloimmunization also happens when an Rh-negative woman undergoes an Rh-positive blood transfusion[1]. The minimal fetal blood volume required to cause alloimmunization varies from 0.1 mL to 1 mL and is possibly associated with the Rh-positive RBCs' immunogenic capacity and the patient's immune responsiveness[2]. Fetomaternal hemorrhage adequately induces alloimmunization. It occurs most commonly at parturition, known as the most vulnerable period, from 15% to 50% of deliveries[3]. When fetomaternal hemorrhage occurs, ectopic pregnancy, threatened abortion, spontaneous or induced pregnancy termination, invasive intrauterine procedures, blunt abdominal trauma, any antepartum bleeding episode and external cephalic version[2,3]. It was determined that if the prevention with anti-D prophylaxis is not performed during the antepartum and within 72 hours of delivery, approximately 14% of these patients will develop anti-Rh antibodies within six months or during their subsequent pregnancy[4]. Hemolytic disease of the fetus and newborn (HDFN) remains a severe pregnancy complication that continues to be a major cause of adverse perinatal outcomes. HDFN is caused by maternal immunoglobulin G (IgG) red cell alloantibodies that are actively transported across the placenta, bind to fetal erythrocytes via the involved antigen, and cause immune-mediated hemolysis and anemia. If left untreated, they may cause fetal heart failure, fetal hydrops, and fetal death[5]. The use of anti-D prophylaxis has led to a decrease in the incidence of Rh alloimmunization in developed countries. About 1.8% of Rh-negative women develop anti-Rh antibodies following only postpartum prophylaxis, and 0.2% of Rh-negative patients develop these antibodies following both antepartum and postpartum prophylaxis[4,6]. However, no immunoprophylaxis has been produced to inhibit non-D alloimmunizations[7].

As a consequence of extended use of anti-D prophylaxis in developed countries, non-D antibodies account for a relatively higher proportion of alloimmunized pregnancies[8]. Previous data indicated that RBC transfusion is the most significant independent risk factor for non-D Rh alloimmunization, followed by delivery, major surgery, and hematological diseases[9]. A limited number of studies examined the management and neonatal outcome of maternal alloimmunization based on the antibody types. This is especially concerning since middle cerebral artery (MCA) peak systolic velocity is the measurement used in routine practice to evaluate fetal anemia. Some patients have multiple RBC antibodies, which might lead to a more complicated state and require additional interventions, including intrauterine transfusion (IUT), during HDFN management in pregnancy than the presence of a single RBC antibody[10].

This study investigates the distribution of antibodies that cause HDFN and compares the clinical outcomes of pregnancies affected by anti-D and anti-D combined with non-D Rh alloimmunization in a Turkish tertiary referral center.

Materials and Methods

This retrospective case-control study was performed in the Kanuni Sultan Süleyman Training and Research Hospital from October 2015 to December 2018. All Rh-negative pregnant women with RBC alloimmunization confirmed by Rh titers, aged between 18 and 40 years, who managed and delivered in this hospital were included in this study. We searched and obtained the perinatal and neonatal data of patients with anti-D antibodies and anti-D combined with non-D Rh antibodies [anti-c, -C, -e, -E, and -Kell (K)] during the study course from the hospital's electronic database and medical files of both the mother and the newborn. The ethics committee of the hospital approved the study (2019/04/86).

Of the 153 pregnant women included in the study, we enrolled 128 patients with anti-D antibodies as the anti-D group and 25 patients with anti-D combined with non-D Rh antibodies as the anti-D combined non-D group. Patients were enrolled only if non-D Rh antibodies occurred in conjunction with an anti-D antibody during the pregnancy course. Patients with multiple pregnancies, any major structural fetal abnormality on the ultrasound scan (US), who delivered at another institution, with unavailable or incomplete medical records, and were unwilling to participate in this study, were excluded. Patients were excluded if fetal or neonatal death occurred for reasons other than alloimmunization. Also, alloimmunized patients were excluded if the antibodies identified were deemed clinically insignificant, including passive anti-D, anti-HLA, anti-N, Ig-M class anti-M, and anti-Le[11].

The following protocol was used to investigate and manage the Rh-sensitized pregnancies in our hospital. All Rh-negative pregnant women were routinely screened with an Rh-positive father for antibodies during the first trimester. The maternal antibody titer was determined utilizing the Indirect Coombs test (ICT). Maternal antibody detection and titrations were conducted by the indirect gel antiglobulin technique. Titers were obtained in the same laboratory since variations in titer results from different laboratories are common. Titrations were determined every 2 to 4 weeks with the exception of anti-K. Anti-K is demonstrated to suppress fetal erythropoiesis, and therefore, antibody titers are not predictive of fetal outcome in HDFN. When anti-K was detected, no more titers were conducted[12]. A titer ≥1:16 indicates a significant risk for HDFN. If the cut-off value was reached, the laboratory follow-up was discontinued. In patients with an Rh-titer of ≥1:16, antenatal fetal monitoring by color Doppler US was performed to determine the MCA peak systolic velocity. Pregnancies complicated by HDFN were managed by weekly monitoring with MCA Doppler US until anemia is suspected and IUT is

required. Suspected fetal anemia requiring IUT was defined as abnormal MCA Doppler US findings and/or the presence of other anemia signs at US (hydrops, cardiomegaly)[13]. We labeled abnormal MCA Doppler US as a peak systolic velocity >1.5 multiples of the median (MoM) value for the gestational age[14]. Signs of fetal hydrops on US were described as elevated fluid in higher than two fetal compartments, including pericardial effusion, pleural effusion, ascites, increased amniotic fluid index, and skin edema[11]. Fetal hydrops was classified as mild or severe. The presence of a distinct rim of ascites with or without pericardial effusion is described as mild fetal hydrops. Fetal hydrops was considered severe when ascites was abundant with the presence or absence of pleural effusion, skin edema, and pericardial effusion[13]. Cordocentesis was performed to confirm fetal anemia if MCA peak systolic velocity exceeded 1.5 MoM and/or if fetal anemia signs were detected on the US. A fetal hematocrit of less than 30% was used as the cut-off for fetal anemia to indicate an IUT[15]. After the procedure, antenatal monitoring was performed by weekly MCA peak systolic velocity measurement and fetal biophysical profile. The time interval between the two transfusions depended on the MCA peak systolic velocity measurements during the follow-up and posttransfusion serum hemoglobin concentrations. Since the positive predictive value for a cut-off value of 1.5 MoM decreased significantly from the first IUT to the second and third IUT, a threshold of 1.73 MoM was used to diagnose fetal anemia at the time of the second and third IUT[16].

Data on maternal age, gravidity, parity, alloimmunization type, the presence or absence of fetal hydrops, MCA peak systolic velocity values, the gestational week at birth, and neonatal outcomes were recorded. For fetuses with anemia, data was further recorded on the gestational week at the hemolytic disease of the fetus (HDF) diagnosis, the gestational week at cordocentesis, the gestational week at the first IUT, fetal hemoglobin and hematocrit values before and after IUTs, and the number of IUTs. Neonatal outcomes consisted of birth weight, Apgar scores at 1- and 5-minutes, neonatal intensive care unit (NICU) admission, the requirement for phototherapy, exchange transfusion, and top-up transfusion treatments. Phototherapy, exchange transfusion, and top-up transfusion treatments were performed based on the Turkish Neonatal Society guidelines[17]. Neonatal laboratory results were recorded to those collected within 48 hours of birth, including ABO and blood groups, direct antiglobulin (Coombs) test (DAT), hemoglobin and hematocrit values, and serum bilirubin levels (total, direct, indirect). Patients who experienced antibody detection recurring times during the same gestation were enrolled as a single record, and the highest titer was recorded during the pregnancy course. For the anti-D combined non-D group, the titers of all antibody types were recorded and used the highest titer in the analysis. Regarding the women who recorded being pregnant more than once during the study course, each alloimmunized pregnancy was marked as a separate pregnancy case.

The mode of delivery was determined by standard obstetric indications[18]. The primary outcome was the occurrence of HDFN and the overall survival rate of the fetuses. HDFN was defined as fetal hydrops, the need for IUT, intrauterine fetal death, neonatal intensive phototherapy, and neonatal exchange or top-up transfusion. The overall survival rate was based on the live infant number one month after birth.

Statistical Analysis

Differences between categorical variables were analyzed by chi-square test or Fisher's exact test, where appropriate. The factors that may correlate with the outcome non-D antibody positive or not were analyzed independently (univariate analysis) by either Student's t-test or Mann-Whitney U test where applicable. Variables such as the gestational week at diagnosis, birth week, and Apgar scores also compared groups of patients with anti-D groups were performed using the Kruskal-Wallis test. Multiple comparison tests were used to know which groups differ from which others. Univariate and multiple logistic regression analyses and adjusted odds ratios with their confidence intervals were used to define independent risk factors for non-D antibody positive. Diagnostic powers of variables used to determine non-D antibody positivity are shown with sensitivity, specificity, positive and negative likelihood ratios. The correlation between binary variables was investigated using the Phi correlation coefficient. Statistical analyses were done using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA), and significance was assumed for a p-value of <0.05.

Results

During the study period from October 2015 to December 2018, a total of 37,344 deliveries occurred at the obstetric unit of the University of Health Sciences Turkey, Kanuni Sultan Süleyman Training and Research Hospital. A total of 178 alloimmunized pregnancies were detected from the medical records of the patients. We excluded 25 patients from this study based on missing medical records or applying the exclusion criteria. Finally, a total of 153 alloimmunized pregnant women and their fetuses were included in this study. None of them were multiple pregnancies. The incidence of pregnancies affected by Rh alloimmunization was 0.40% (153/37344), of which 0.34% (128/37344) of them were alloimmunization with anti-D antibody and 0.06% (25/37344) with anti-D combined non-D Rh antibodies.

Table 1 presents the maternal demographic characteristics, the course of affected pregnancies, management, and treatment outcomes of neonates stratified by anti-D antibody group and anti-D combined with non-D Rh antibodies group. Table 2 summarizes the prenatal and postnatal characteristics of the anti-D combined non-D group. Anti-ce was most common (13/25, 52%), followed by anti-Cce (5/25, 20%), anti-Ce

Table 1. Maternal demographic characteristics, the course of affected pregnancies, management, and treatment outcomes of neonates stratified by the anti-D antibody group and the anti-D combined with non-D Rh antibodies group

		Anti-D combined non-D group	Anti-D group	p
Gravidity		4 (2-5)	3 (2-4)	0.422
Parity		2 (1-3)	2 (1-3)	0.463
Previous abortion		0.56±0.96	0.54±1.10	0.833
Fetal gender	Male	10 - (40.00)	75 - (58.60)	0.123
	Female	15 - (60.00)	53 - (41.40)	
Gestational week at diagnosis		31.04±4.98	32.16±4.96	0.201
MCA Doppler US	A Zone (>1.5 MoM)	5 - (20.00)	3 - (2.30)	<0.001*
	B Zone (1.29-1.5 MoM)	3 - (12.00)	5 - (3.90)	
	C Zone (<1.29 MoM)	17 - (68.00)	120 - (93.80)	
Fetal hydrops		3 - (12.00)	1 - (0.78)	0.014*
First indirect Coombs test	16-256	9 - (36.00)	83 - (64.84)	<0.001
	512-8192	8 - (32.00)	40 - (31.25)	
	≥16384	8 - (32.00)	5 - (3.90)	
Last indirect Coombs test	16-256	7 - (28.00)	73 - (57.03)	<0.001
	512-8192	7 - (28.00)	47 - (36.71)	
	≥16384	11 - (44.00)	8 - (6.25)	
Intrauterine transfusion		16 - (64.00)	7 - (5.46)	<0.001*
Gestational week at cordocentesis and first intrauterine transfusion		27 (21-33)	30 (25-32)	0.093
Cesarean delivery		20 - (80.00)	75 - (58.59)	0.070
Birth week		34.64±4.27	37.50±1.92	<0.001
Birth weight		2450.80±832.36	3029.49±536.54	<0.001
1-min Apgar score		6.20±2.48	7.24±1.27	0.085
5-min Apgar score		7.52±3.07	9.02±0.91	0.013
NICU admission		17 - (77.2)	53-(43.4)	<0.001
NICU admission, days		16.58±9.89	11.32±9.31	0.014
NICU admission, days	No	8 - (32.00)	75 - (58.59)	0.002
	≤7 days	2 - (8.00)	21 - (16.40)	
	>7 days	15 - (60.00)	32 - (25.00)	
NICU admission indications	RDS	3 - (17.64)	19 - (38.45)	0.173*
	Jaundice	14 - (82.35)	25 - (48.07)	
	Sepsis	0 - (0.00)	5 - (9.61)	
	Hypoglycemia	0 - (0.00)	2 - (3.84)	
Hemoglobin value at birth		14.81±6.62	16.03±2.42	0.007
Hematocrit value at birth		45.87±23.28	47.51±7.02	0.030
Total bilirubin value at birth		9.82±6.17	11.34±7.89	0.727
Phototherapy		15 - (68.18)	30 - (23.62)	<0.001

Neonatal exchange transfusion	4 - (18.18)	4 - (3.14)	0.017*
Top-up transfusion	6 - (27.27)	2 - (1.57)	**<0.001***
Direct Coombs test positivity	13 - (59.09)	32 - (25.19)	**0.002***
In utero fetal demise	3 - (12.00)	1 - (0.08)	**0.014***
HDFN	18 - (72.00)	31 - (24.21)	**<0.001**

Data were presented as median (minimum-maximum), mean ± standard deviation, and n (%).
*represents Fisher's exact test's p-value

(3/25, 12%), anti-cE (1/25, 4%), anti-Cc (1/25, 4%), anti-E (1/25, 4%), and anti-K (1/25, 4%). The patients in the anti-D group had a similar number of gravidity, parity, and previous abortions with the anti-D combined non-D group. Also, the two groups were comparable regarding fetal gender and the gestational week at the time of diagnosis. All the pregnant women who underwent cordocentesis had anemic fetuses and experienced intrauterine transfusion. The median gestational week at cordocentesis and the first IUT was similar between the groups. The severe fetal hydrops rate was significantly higher in the anti-D combined non-D group (3/25, 12%) than in the anti-D group (1/128, 0.08%, p<0.001). All fetuses with severe hydrops received an intrauterine transfusion in both groups. In the anti-D combined non-D group, 100% (n=3) of severe fetal hydrops cases resulted in fetal death during the pregnancy course. Two of these had anti-D combined with anti-ce and ended in fetal death at the 25th and 28th weeks of gestation. One had a combination of anti-D and anti-cE and resulted in fetal death in the 22nd week of pregnancy. In the anti-D group, a severe fetal hydrops case was born by cesarean delivery at 30th weeks of gestation due to non-reassuring fetal heart rate but did not survive after the delivery. The IUT requirement in the anti-D combined non-D group (16/25, 64%) tended to be significantly higher than that in the anti-D group (5/128, 7.46%, p<0.001). No intraperitoneal transfusion was performed. The cesarean delivery rate was not significantly different between the anti-D group (58.59%) and the anti-D combined non-D group (80%, p=0.070). The gestational week at birth and birth weight in the anti-D combined non-D group (34.64±4.27 weeks and 2450.80±832.36 g, respectively) were significantly lower than that of the anti-D group (37.50±1.92 weeks and 3029.49±536.54, respectively, p<0.001).

Regarding the management of the neonates, 10.7% (16/149) of infants required treatments for anemia. The incidence of neonatal exchange transfusion in the anti-D combined non-D group (4/25, 18.18%) was significantly higher than in the anti-D group (4/128, 3.14%, p=0.017). The top-up transfusion requirement in the anti-D combined non-D group (6/22, 27.27%) tended to be significantly higher than that in the anti-D group (2/152, 1.57%, p<0.001). The postnatal phototherapy frequency was significantly higher in the anti-D combined non-D group (15/22, 68.18%) than in the anti-D group (30/152, 23.62%, p<0.001). In total, 46.9% (70/149) of neonates required NICU admission. The NICU admission rate was significantly higher in the anti-D combined non-D group (17/22, 77.2%) than in the anti-D group (53/152, 43.4%, p<0.001). The duration of NICU admission in the anti-D combined non-D group (16.58±9.89 days) was significantly longer than in the anti-D group (11.32±9.31 days, p=0.014). A DAT was performed on all the neonates; 59.09% (13/22) were positive in the anti-D combined non-D group, and 25.19% (32/149) were positive in the anti-D group (p=0.002). A total of 38.5% (59/153) of the fetuses were affected by maternal Rh alloimmunization and developed HDFN. The frequency of HDFN in the anti-D combined non-D group (18/25, 72%) was significantly higher than that in the anti-D group (31/128, 24.2%, p<0.001). All newborns in the anti-D combined non-D group had survived one month after birth.

The requirement of IUT and phototherapy had a high sensitivity to determine the non-D alloantibody positivity (0.64 and 0.68, respectively). However, the presence of fetal hydrops, exchange transfusion, top-up transfusion, and in utero fetal demise had a low sensitivity to determine the non-D alloantibody positivity (0.12, 0.18, 0.27, and 0.12, respectively). Therefore, it is not reasonable to make predictions about the non-D alloantibody positivity with these parameters. However, all parameters had moderate and high specificity values in detecting the absence of non-D alloantibody. Table 3 shows the positive and negative likelihood ratios of these parameters.

Table 4 provides the correlation coefficients for non-D alloantibody positivity of key parameters, and Table 5 presents the results of the univariate and multiple logistic regression analysis. Only the requirement of IUT was significant in the final model of logistic regression analysis, which was established with all variables found to be significant in the univariate analysis. In the presence of the IUT requirement, the risk of non-D alloantibody positivity increased 21.4 times and was statistically significant (p<0.001).

Discussion

The current study evaluates the clinical outcomes of pregnancies affected by anti-D and anti-D combined with non-D Rh antibodies. Our study indicates that anti-D combined with another RBC alloantibody resulted in significantly higher HDFN rates than anti-D alloimmunized pregnancies. Also, anti-D in association with non-D Rh antibodies resulted in more severe HDFN requiring more invasive treatment procedures, including

Table 2. The prenatal and postnatal characteristics of the anti-D combined non-D group

Rbc antibody		Gestational week at critical titer reached	Maximum. maternal antibody titer	IU Tx, n NICU admission, days	Gestational week at delivery indication for NICU admission	Neonates				
						Phototherapy	Exchange Tx	Top-up Tx		
	1	29	1/4096	2	35+4	10	RDS	+		
	2	35	1/8192	-	35+2	15	Jaundice	+		+
Cce (n=5)	3	29	1/65536	3	37	30	Jaundice	+		+
	4	32	1/2048	4	36	20	Jaundice	+		+
	5	30	1/32768	3	35	3	Jaundice	+		
	1	25	1/256	-	38	-				
	2	31	1/32768	3	35	13	Jaundice	+		
	3	32	1/16384	2	25 (IUFD)					
	4	31	1/32768	5	34+1	12	Jaundice	+	+	
	5	30	1/65536	2	34	42	Jaundice	+		+
	6	19	1/65536	2	34+2	13	Jaundice	+		
Ce (n=13)	7	29	1/1024	1	33+3	10	Jaundice	+		
	8	25	1/32768	1	27+6	25	RDS (died)			
	9	33	1/8192	1	33+2	25	RDS			
	10	30	1/32768	3	33+1	25	Jaundice	+		+
	11	35	1/32	-	37+6	-				
	12	32	1/128	5	35+5	9	Jaundice	+		
	13	31	1/4096	-	37+4	7	Jaundice	+	+	
	1	35	1/256	-	37	-				
Ce (n=3)	2	29	1/32768	7	34+4	15	Jaundice	+	+	
	3	35	1/32768	-	37+2	-				
cE (n=1)	1	22	1/4096	-	22 (IUFD)	-				
Cc (n=1)	1	31	1/65536	4	35+6	9	Jaundice	+	+	
E (n=1)	1	33	1/64	-	37	-				
Kell (n=1)	1	32	1/64	-	38	-				

IUT, neonatal exchange transfusion, or top-up transfusion.

We detected an RBC alloimmunization incidence of 0.4% (153/37344) that was similar to that reported by previous studies (0.4%-1.1%)[11,19,20]. Also, the non-D Rh alloimmunization incidence in our study was 0.06% (25/37344), which is considerably lower than the 0.32% Koelewjin et al.[21] found in the Netherlands, the 0.16% Gotvall and Filbey[22] found in Sweden, and slightly higher than the 0.04% Healsmith found in Australia[8]. The distribution of maternal alloimmunization and HDFN with anti-D and non-D antibodies varies in different populations and countries. This difference can be explained by the geographic variations of Rh antigen frequencies in the populations examined, transfusion practices, and antibody screening frequencies in different countries[8,11]. According to the blood transfusion policy in Turkey, ABO and Rh blood types are routinely identified before blood transfusion. Pretransfusion, an extended Rh antigen phenotyping, is only performed if the patient has previously detected antibodies or a patient who may require a long-term transfusion regimen.

Nordvall et al.[23] reported that the combination of antibody specificities was more harmful and brought about a more severe form of HDFN than single antibody specificities. They suggested that increased binding of multiple antibodies on target RBCs led to higher hemolysis levels due to a synergistic effect. Markham et al.[24] also stated that multiple RBC antibodies are related to an increased risk for significant HDFN development and proposed two possible theories. The first theory is the cumulative effect involving increased hemolysis due to the binding of the multiple antibodies to more fetal RBCs. The second theory is a more aggressive immune response in patients prone to developing

Table 3. Diagnostic performance of key parameters for non-D alloantibody positivity

Anti-D combined non-D group vs fetal hydrops	
Sensitivity (95% CI)	0.12 (0.041-0.29)
Specificity (95% CI)	0.99 (0.95-0.99)
Positive likelihood ratio (95% CI)	15.3 (1.66-141.73)
Negative likelihood ratio (95% CI)	0.88 (0.76-1.026)
Anti-D combined non-D group vs intrauterine transfusion	
Sensitivity (95% CI)	0.64 (0.44-0.79)
Specificity (95% CI)	0.94 (0.89-0.97)
Positive likelihood ratio (95% CI)	11.70 (5.36-25.47)
Negative likelihood ratio (95% CI)	0.38 (0.22-0.64)
Anti-D combined non-D group vs phototherapy	
Sensitivity (95% CI)	0.68 (0.47-0.83)
Specificity (95% CI)	0.76 (0.68-0.82)
Positive likelihood ratio (95% CI)	2.88 (1.89-4.40)
Negative likelihood ratio (95% CI)	0.41 (0.22-0.77)
Anti-D combined non-D group vs exchange transfusion	
Sensitivity (95% CI)	0.18 (0.07-0.38)
Specificity (95% CI)	0.96 (0.92-0.98)
Positive likelihood ratio (95% CI)	5.77 (1.55-21.39)
Negative likelihood ratio (95% CI)	0.84 (0.69-1.03)
Anti-D combined non-D group vs top-up transfusion	
Sensitivity (95% CI)	0.27 (0.13-0.48)
Specificity (95% CI)	0.98 (0.94-0.99)
Positive likelihood ratio (95% CI)	17.31 (3.73-80.37)
Negative likelihood ratio (95% CI)	0.73 (0.57-0.95)
Anti-D combined non-D group vs in utero fetal demise	
Sensitivity (95% CI)	0.12 (0.04-0.29)
Specificity (95% CI)	0.99 (0.95-0.99)
Positive likelihood ratio (95% CI)	15.36 (3.56-45.39)
Negative likelihood ratio (95% CI)	0.89 (0.45-0.98)

CI: Confidence interval

multiple RBC antibodies. In the second theory, women prone to developing multiple antibodies have a more aggressive immune response. This theory may also explain the increased risk of significant HDFN in the setting of multiple antibodies, but only one corresponding fetal or neonatal antigen[24].

Previous studies reported that the presence of anti-D combined with another RBC antibody resulted in a significantly increased risk of developing HDFN and receiving invasive treatment procedures, including IUT, top-up transfusion, or exchange transfusion[10,23,24]. Also, these studies stated that the majority

Table 4. Correlation coefficients for non-D alloantibody positivity of key parameters

	Anti-D combined non-D group (Phi coefficient and p-value)
Fetal hydrops	0.260 (p=0.001)
Intrauterine transfusion	0.606 (p<0.001)
Phototherapy	0.344 (p<0.001)
Exchange transfusion	0.237 (p=0.004)
Top-up transfusion	0.404 (p<0.001)
In utero fetal demise	0.260 (p=0.001)

of alloimmunizations with multiple antibodies included anti-D and the presence of anti-D in multiple antibody combinations was more likely to develop significant HDFN requiring invasive treatment methods than those of the other combinations. However, Sharma et al.[25] reported a rare case in which the neonate presented severe hyperbilirubinemia and jaundice due to anti-C and anti-e alloimmunization. They suggested that DCT should be performed in all neonates with severe jaundice even when there is no ABO and Rh isoimmunization. Anti-C can be additive to the hemolytic effects of other antibodies and is more often related to severe outcomes in pregnancies complicated by multiple antibodies or in compound antibodies[8,25,26]. All the other non-D Rh antibodies may cause adverse neonatal outcomes[8]. However, we only included these antibodies in our study when they are present in conjunction with anti-D to maintain the focus on the additive effects of these antibodies. Currently, all patients with alloimmunization are managed as anti-D alloimmunization based on the various published data about this complication without predicting whether the fetal and neonatal outcomes are similar and whether this approach is correct[10].

Management of Rh-isoimmunized pregnancies relies on the regular monitoring of maternal antibody concentration via calculating antibody titration for most antibodies[12]. Antibody titration studies evaluate the antibody quantity and serve as a screening test to indicate when MCA peak systolic velocity measurement with Doppler US should be initiated. MCA peak systolic velocity above 1.5 MoM can predict moderate to severe fetal anemia with a sensitivity of 100% and a false positive rate of 12%[2]. In pregnancies with Rh alloimmunization, after the occurrence of fetal anemia, the antibody titer should not be used to predict the risk of severe HDFN[27]. Nevertheless, previous reports determined a critical titer of ≥16–32 by conventional tube testing. Below this range, no severe adverse outcomes were observed, including a requirement for IUT, intrauterine fetal demise, or stillbirths[11]. Fink et al.[28] reported that the indirect gel antiglobulin technique might perform similar to the conventional tube testing in titrating alloantibodies to Rh antigens. Up to now, few studies have evaluated the correlation of HDFN with the gel titer cut-off value. In our study, across

Table 5. The results of univariate and multiple logistic regression analysis

	Univariate logistic regression			95% Confidence intervals		p
Variables	B	S.E.	OR	Lower Limit	Upper Limit	
Fetal hydrops	2.852	1.178	17.318	1.722	174.120	**0.015**
Intrauterine transfusion	3.425	0.570	30.730	10.058	93.891	**<0.001**
Phototherapy	1.936	0.503	6.929	2.584	18.575	**<0.001**
Exchange transfusion	1.922	0.751	6.833	1.569	29.765	**0.010**
Top-up transfusion	3.154	0.859	23.437	4.356	126.107	**<0.001**
In utero fetal demise	2.852	1.178	17.318	1.722	174.120	**0.015**
	Multiple logistic regression			**95% Confidence intervals**		
Variables	**B**	**S.E.**	**OR**	**Lower limit**	**Upper limit**	**p**
Intrauterine transfusion	3.067	0.616	21.488	6.430	71.809	**<0.001**

all antibody types, 52.2% (n=80) of the patients did not exceed the critical titer of 16 with the gel technique, and no neonates born to these mothers with a titer ≤16 met the HDFN criteria, suggesting that this cut-off value was clinically suitable.

Study Limitations

The main strength of this study was that it was conducted in a well-organized tertiary center with trained medical staff who delivered adequate health care to alloimmunized pregnant patients. Indirect gel antiglobulin testing was used to validate the critical antibody titer. However, there are some limitations to this study. This study was designed retrospectively, with the potential to contain study limitations. Also, patients' previous history of RBC transfusions was not identified due to the lack of data. The rarity of non-D Rh antibodies resulted in the low sample size of this study. The differences in neonatal outcomes between specific single and multiple antibodies could not be identified.

Conclusion

The incidence of Rh alloimmunization has decreased notably in recent decades, most probably due to the extended use of anti-D prophylaxis and non-D antibodies. These antibodies represent a relatively higher proportion of alloimmunized pregnancies. Anti-D combined with another non-D Rh alloantibody resulted in significantly higher HDFN rates than anti-D alloimmunized pregnancies. Also, anti-D in association with non-D Rh antibodies resulted in more severe HDFN requiring more invasive treatment procedures, including IUT, neonatal exchange transfusion, or top-up transfusion.

Ethics

Ethics Committee Approval: The ethics committee of the hospital approved the study (2019/04/86).
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Z.G.Ö., Concept: Z.G.Ö., S.C.O., Design: Z.G.Ö., S.C.O., Data Collection or Processing: Z.G.Ö., Analysis or Interpretation: Z.G.Ö., S.C.O., Literature Search: Z.G.Ö., S.C.O., Writing: Z.G.Ö., S.C.O.
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Neoadjuvant chemotherapy for locally advanced stage (IB2-IIA2-IIB) cervical carcinoma: Experience of a tertiary center and comprehensive review of the literature

Lokal olarak ileri evre (IB2-IIA2-IIB) servikal karsinom için neoadjuvan kemoterapi: Tersiyer merkez deneyimi ve literatürün kapsamlı incelemesi

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Abstract

Objective: This study aimed to evaluate neoadjuvant chemotherapy (NACT) for locally advanced stage cervical carcinoma.

Materials and Methods: Data of 43 patients with locally advanced cervical carcinoma who had NACT were reviewed. NACT protocols implemented included cisplatin/5-fluorauracil, cisplatin/UFT, and carboplatin/paclitaxel. After NACT, the patients were re-examined, and patients who had a tumor size ≤40 mm underwent Piver-Rutledge type III radical hysterectomy, while other patients received radiotherapy. Following NACT, clinical responses were assessed according to the criteria of the World Health Organization.

Results: The mean age of the patients was 49.4 years, and the median follow-up duration was 48 (range, 5-228) months. The median tumor sizes were 50 and 30 mm before and after NACT, respectively. Complete clinical response was observed in 4 (9.3%) patients, partial clinical response in 8 (18.6%), and pathologic complete response in 3 (6.9%). Stable disease was noted in 30 (69.9%) patients and progression in 1 (2.3%) patient. After NACT, 31 patients have undergone radical surgical procedures. The 5-year disease-free survival rate was 72%, and the 5-year disease-specific survival rate was 91%. Age, International Federation of Gynaecology and Obstetrics 2009 stage, histopathologic type, NACT protocol, rate of decrease in tumor size after NACT, clinical response, number of courses, tumor size before NACT, tumor size after NACT, and lymph node metastasis were not associated with disease-free survival.

Conclusion: Following NACT, a significant reduction in tumor dimension was observed, and the probability of radical surgery is increased. However, clinical response was not predictive of survival.

Keywords: Cervical carcinoma, locally advanced stage, neoadjuvant chemotherapy, survival

Öz

Amaç: Bu çalışmada neoadjuvan kemoterapinin (NACT) etkinliği değerlendirilmiştir. NACT, lokal olarak ilerlemiş servikal karsinomda başlangıç tedavisi için yöntemlerden biridir.

Gereç ve Yöntemler: Lokal olarak ilerlemiş servikal karsinomlu, NACT olan 43 hastanın verileri gözden geçirildi. NACT protokolleri sisplatin/5-fluorourasil, sisplatin/UFT ve karboplatin/paklitaksel idi. NACT sonrası hastalar tekrar muayene edildi ve tümör boyutu 40 mm ve altı olan hastalar ameliyat edildi (Piver-Rutledge tip III radikal histerektomi) ve diğer hastalara radyoterapi verildi. NACT klinik yanıtı, Dünya Sağlık Örgütü kriterlerine göre değerlendirildi.

Bulgular: Hastaların ortalama yaşı 49,4 yıl ve ortanca takip süresi 48 (aralık, 5-228) aydı. Ortalama tümör boyutu NACT'den önce 50 mm ve NACT'den sonra 30 mm idi. Yanıt oranları aşağıdaki gibidir; dört hastada (%9,3) tam klinik yanıt, sekiz hastada (%18,6) kısmi klinik yanıt ve üç hastada (%6,9) patolojik tam yanıt. Otuz hastada (%69,9) stabil hastalık ve bir hastada (%2,3) progresyon görüldü. NACT'den sonra 31 hasta radikal cerrahi prosedür geçirdi. Beş yıllık hastalıksız sağkalım %72,5 yıllık hastalığa özgü sağkalım %91 idi. Yaş, Uluslararası Jinekoloji ve Obstetrik Federasyonu 2009 evresi,

PRECIS: This study evaluated the outcomes of neoadjuvant chemotherapy in cervical carcinoma.

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histopatolojik tip, NACT protokolü, NACT sonrası tümör boyutundaki azalma oranı, klinik yanıt, kurs sayısı, NACT öncesi tümör boyutu, NACT sonrası tümör boyutu ve lenf nodu metastazı hastalıksız sağkalım ile ilişkili değildi.
Sonuç: NACT sonrası tümör boyutunda önemli bir azalma oldu ve radikal cerrahi olasılığı arttı. Ancak klinik yanıt, sağkalımı tahmin etmedi.
Anahtar Kelimeler: Serviks kanseri, lokal ileri evre, neoadjuvant kemoterapi, sağkalım

Introduction

Cervical carcinoma (CC) is the fourth most common cancer in women worldwide, and it is the fourth leading cause of cancer-related deaths[1]. The type of treatment type is based on the disease stage. Surgery, radiotherapy (RT), and chemotherapy (CT) have been suggested as standard treatment approaches[2]. The effectiveness of radical hysterectomy (RH) and RT in early-stage CC is comparable[3]. Owing to the preservation of ovarian activity, having lesser sexual dysfunction in surgery than in RT, and leaving RT as an alternative treatment for recurrence, surgery is currently the preferred method of treatment CC. However, after RH, the need for RT increases. Landoni et al.[4] reported that 84% of patients with stage IB-IIA disease received postoperative RT.

Concurrent chemoradiation used to enhance the effect of RT on the treatment of recurrence and locally advanced CC improves the response rate and survival of the patients[5]. This treatment modality not only controlled the course of a localized tumor but also decreased distant metastasis; thus, neoadjuvant chemotherapy (NACT) becomes a current issue. The main objectives of NACT are to eliminate micrometastasis, make the tumor smaller enough for surgical removal, and increase the survival of patients following the RF or RT. However, RT following NACT (sequential RT) had no effect on survival[6,7] and even worsened it[8,9]. These negative results are explained by the cross-resistance between the two treatment modalities and intracellular alterations[10]. By contrast, the cross-resistance problem does not exist in RH and the residual tumor is removed. Therefore, RF following the NACT is expected to increase patient survival. In a meta-analysis of 21 phase III trials, NACT followed by RH improved overall survival (OS) by 14% in comparison with RT alone[11]. However, in a study of the gynecologic oncology group (GOG), compared with NACT followed by RH, RH alone did not show any improvement[12].

The value of NACT in the treatment of CC is not appropriately defined until now; especially, in early-stage CC, uncertainty is much more common. Thus, this study aimed to evaluate the effect of NACT on the outcomes of patients with locally advanced CC (stage IB2, IIA2, and IIB).

Materials and Methods

Medical records of patients with stage IB2, IIA2, or IIB CC between 1998 and 2020 were reviewed retrospectively. This study included 43 patients who received NACT. These patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO 2009) staging system. In all patients, diseases were staged using upper abdominal tomography, pelvic magnetic resonance imaging (intravenous pyelography as needed), and gynecologic examination under general anesthesia. Histopathological evaluation was carried out according to the 2014 World Health Organization (WHO) criteria[13]. This study was approved by the local ethical committee (file no. 90057706-799/08; 05.06.2020).

Cisplatin/5-fluorouracil (5-FU) (CF), cisplatin/UFT™ (CU), and carboplatin/paclitaxel (CbP) combinations were applied as NACT protocols. The CF protocol started with cisplatin at a dose of 75 mg/m^2, given as an infusion within 1 h, followed by 5-FU at a dose of 500 mg/m^2 given within 6 h. The 5-FU dose was repeated at days 2-5 of the protocol. CT was given at 28 days intervals. The CU protocol was started with cisplatin at a dose of 75 mg/m^2, given as an infusion within 1 h. UFT™ [urasil (224 mg)-tegafur (100 mg) capsule, Bristol-Myers Squibb, NY, USA] was started at the same day as one capsule administered orally for 14 days. CT was given at 21 days intervals. The CbP protocol started with paclitaxel at a dose of 175 mg/m^2, given as an infusion within 3 h, followed by carboplatin dose calculated by using an area under curve of 6 (maximum dose of 750 mg) given within CT, the following criteria were supplied: (i) performance status ≤2 according to the Eastern Cooperative Oncology Group standards, (ii) adequate bone marrow function (leukocytes ≥3.000/mL, neutrophils ≥1.500/mL, platelets ≥100.000/mL, and hemoglobin ≥10 mg/dL), (iii) adequate hepatic function (total bilirubin, alanine aminotransferase, and aspartate aminotransferase levels were below twice of the upper limits), and (iv) adequate renal function (glomerular filtration rate >60 mL/min). Patients were evaluated for CT toxicity and adjustment of the next dose based on the complete blood counts and biochemical tests at every 10 days. Toxicity was assessed according to the WHO criteria[14].

Patients were re-examined under general anesthesia after two or three cycles of NACT. Patients who had tumors ≤40 mm underwent to Piver-Rutledge type III hysterectomy, while other patients received RT. Adjuvant treatment decisions for all patients were made by a gynecologic oncology council after RH. Patients with high-risk status received postoperative RT. Up until 2001, the criterion for postoperative adjuvant RT was the presence of at least one of the major risk factors (i.e., positive lymph nodes, parametrial involvement, presence of a tumor within the surgical margins, and tumor size ≥4 cm) or two of the minor risk factors (i.e., lymphovascular space invasion, stromal invasion of ≥1/2, tumor size 2-4 cm, and ≥3 lymph nodes with microscopic metastasis). After 2001, only patients with positive lymph nodes and/or parametrial involvement and/or a tumor within the surgical margins received adjuvant RT. RT was administered alone or in combination with CT (concurrent chemoradiation).

Following the CT, clinical responses were assessed according to the WHO criteria[14]: complete clinical response (CCR), absence of gross tumor; partial clinical response (PCR), >50% decrease in tumor size; stable disease (SD), <50% decrease or <25% increase in tumor size; progressive disease (PD), >25% increase in tumor size or new tumor foci were found. The absence of tumor in the pathology specimen (RH, ovaries, and lymph nodes) was defined as pathologic complete response (Pat CR).

Patients were followed by a pelvic examination, vaginal smear, abdominal ultrasonography, whole blood count, and blood biochemistry tests in the first 2 years after treatment in every 3 months, every 6 months up to the fifth year, and then once a year. Chest X-ray imaging was requested annually or when clinically suspicious recurrence was detected. Advanced imaging techniques (computed tomography, magnetic resonance imaging, or positron emission tomography-computed tomography) were performed when necessary. If recurrence was detected during follow-up, the time and site of recurrence were recorded. Deaths were also recorded.

The time from the first dose of NACT to any cause of death because of disease or last follow-up visit was defined as OS. The time from the first dose of NACT to death because of the disease or last follow-up visit was defined as disease-specific survival (DSS). Disease-free survival (DFS) was defined as the period from the first dose of NACT to confirmed recurrence or refractory disease with clinical examination and/or radiological imaging or the period from the initial surgery to the last follow-up visit in those who did not develop refractory/recurrence disease.

Statistical Analysis

SPSS 20.0 (SPSS Inc., Chicago, IL) was used for data review and statistical analysis. Descriptive statistics were expressed as mean ± standard deviation and median (minimum-maximum) for continuous variables and n (%) for categorical variables. The defining effect of surgical-pathologic factors on clinical response was assessed using the chi-square test. The Kaplan-Meier method was used to evaluate survival results. Survival curves were compared in the log-rank test. Significance was defined as $p<0.05$.

Results

The mean age of the patients was 49.4±8.67 (range, 33-70) years. According to the FIGO 2009 staging system, 28 (65.1%) patients had stage IB_2 disease, 11 (25.6%) had stage IIA2 disease, and 4 (9.3%) had stage IIB disease. Histopathologic diagnosis was squamous cell carcinoma in 39 (90.7%) patients. The median tumor size was 50 mm (range, 30-70 mm) before NACT and 30 mm (range, 0-70 mm) after NACT. In one patient, the tumor size was <40 mm before NACT, although this patient had stage IIB disease. As a NACT regimen, 36 (83.7%) patients received CF, 3 (7%) received CbP, and 4 (9.3%) received CU. Moreover, 27 (62.8%) patients received three cycles of CT and 16 (37.2%) received two cycles (Table 1).

The control treatment after NACT showed that the mean tumor size decreased to 32.4±15.26 mm. Moreover, 4 (9.3%) patients obtained CCR. The rate of decrease in tumor size was >25% in 30 (69.8%) patients and >50% in 12 (27.9%) patients. The decrease in tumor size was <25% in six patients, but in 7 (16.3%) patients, there was no change in the tumor size. Accordingly, the calculated overall clinical response (OCR) rate was 27.9% (CCR, 9.3%, n=4; PCR, 18.6%, n=8). The SD rate was 69.9% (n=30), whereas the PD rate was 2.3% (n=1) (Table 1).

After NACT use, a surgical approach was feasible in 31 (72.1%) patients. This rate was 69.4% in patients who received CF and 66.7% in patients who received CbP. Four of the patients who received CU became operable. Finally, after NACT, 31 patients underwent surgery, and Piver-Rutledge type III RH + bilateral salpingo-oophorectomy + para-aortic-bilateral pelvic lymphadenectomy was performed. Moreover 5 (11.7%) of the remaining 12 patients received RT alone or concurrent chemoradiation, and the other 7 (16.3%) patients received concurrent chemoradiation after extraperitoneal/transperitoneal lymph node dissection (Table 1).

The tumor size was <4 cm during clinical examination in all patients who underwent surgery. However, in the postoperative pathological evaluation, the tumor size ranged from 4 to 6 cm in eight patients. After radical surgery of these 31 patients, 6 (19.4%) were found to have parametrial involvement and 1 (3.2%) had surgical border invasion. Lymph node metastasis was evaluated in 38 patients (31 patients underwent RH + lymphadenectomy and seven patients underwent extraperitoneal/transperitoneal lymphadenectomy + RT). Therefore, lymph node metastasis was detected in 17 (44.7%) of these 38 patients. Moreover, 3 of 7 patients who underwent extraperitoneal/transperitoneal lymph node dissection were found to have lymph node metastasis. In the assessment after NACT, three of the four patients who had no tumor in the cervix were also tumor negative after the pathological examination. However, in one patient, although no tumor was seen in the cervix, lymph node metastasis was detected. Consequently, the Pat CR rate was 6.9% (3/43). In addition, 24 (77.4%) of the 31 patients received concurrent chemoradiation after radical surgery. As a result, 36 of the 43 patients received RT (Table 2).

The factors determining clinical response to NACT were investigated. We compared 31 patients who had no clinical response (SD + PD) following NACT with 12 patients who had clinical response (CCR + PCR) following NACT. Age, FIGO 2009 stage, tumor size before NACT, NACT combination, number of NACT cycles, and histopathologic type were found to be not predictive of clinical response ($p>0.05$) (Table 3).

The median duration of follow-up in the entire cohort was 48 (range, 5-228) months. During follow-up, 11 patients had recurrence and six patients died. Three patients died of the disease during the study period. The 5-year DFS rate was 72%,

Table 1. Characteristics of the patients (n=43)

Characteristics		Mean ± SD	Median (range)
Age (years)		49.4±8.67	48 (33-70)
Tumor size before NACT (mm)		54.2±9.81	50 (30-70)
Tumor size after NACT (mm)		32.4±15.26	30 (0-70)
		n	%
FIGO 2009 stage	IB2	28	65.1
	IIA2	11	25.6
	IIB	4	9.3
Tumor size according to FIGO 2018 stage	<2 cm	-	-
	≥2 cm to <4 cm	1	2.3
	≥4 cm	42	97.7
Pathology	Squamous cell carcinoma	39	90.7
	Adenocarcinoma	3	7
	Adenosquamous	1	2.3
NACT protocol	Cisplatin and 5-fluorouracil	36	83.7
	Carboplatin and paclitaxel	3	7
	Cisplatin and UFT™	4	9.3
Number of cycles	2	16	37.2
	3	27	62.8
Tumor size after NACT	Increased	1	2.3
	Not changed	6	14
	Reduce in size of <25%	6	14
	Reduce in size among ≥25% to <50%	18	41.9
	Reduce in size by ≥50% (with gross tumor)	8	18.6
	No gross tumor (clinically)	4	9.3
Clinical response of NACT	Complete clinical response	4	9.3
	Partial clinical response	8	18.6
	Stabile disease	30	69.9
	Progressive disease	1	2.3
Curative intend primary treatment after NACT	Surgery	31	72.1
	Radiotherapy	12	27.9
Curative intend primary treatment after NACT in detailed	Surgery	31	72.1
	Only radiotherapy	2	4.7
	Concomitant chemoradiotherapy	3	7
	Extraperitoneal LND+radiotherapy	6	14
	Transperitoneal LND+radiotherapy	1	2.3

SD: Standard deviation, NACT: Neoadjuvant chemotherapy, LND: Lymphadenectomy, FIGO: Federation of Gynecology and Obstetrics

the 5-year DSS rate was 91%, and the 5-year OS rate was 87% in the study group.

The effects of the clinical and pathological parameters on DFS were evaluated. Age, FIGO 2009 stage, histopathologic type, NACT protocol, treatment after NACT, rate of decrease in tumor size after NACT, clinical response (Figure 1), number of courses, tumor size before NACT, tumor size after NACT, and lymph node metastasis were not associated with DFS (Table 4). Table 5 and 6 represent the clinical response and survival rates of relevant studies investigating the efficacy of NACT in early-

Table 2. Surgical and pathologic characteristics of patients who underwent surgery (n=31 patients)

Characteristics		Mean ± SD	Median (range)
Age (year)		48.7±7.28	48 (33-66)
Tumor size before NACT (mm)		55.5±9.95	50 (30-70)
Pathological tumor size after NACT (mm)		31±16.50	30 (0-60)
Number of removed lymph node[1]		55.1±27.49	51 (11-160)
Number of metastatic lymph node[1]		4±3.98	2.5 (1-15)
		n	%
FIGO 2009 stage	IB2	26	83.9
	IIA2	3	9.7
	IIB	2	6.5
Tumor size according to FIGO 2018 stage	<2 cm	-	-
	≥2 cm to <4 cm	1	3.2
	≥4 cm	30	96.8
Pathology	Squamous cell carcinoma	28	90.3
	Adenocarcinoma	2	6.5
	Adenosquamous	1	3.2
NACT protocol	Cisplatin and 5-fluorouracil	25	80.6
	Carboplatin and paclitaxel	2	6.5
	Cisplatin and UFTTM	4	12.9
Number of cycles	2	11	35.5
	3	20	64.5
Shrinking in tumor size	Tumor size increased	1	3.2
	Tumor size not changed	3	9.7
	<25%	2	6.5
	≥25% to <50%	16	51.6
	≥50%	9	29
Lymph node metastasis[1]	Negative	21	55.3
	Positive	17	44.7
Site of metastatic lymph node[1]	Only pelvic	10	23.3
	Only para-aortic	1	2.3
	Pelvic and para-aortic	3	7
	Not reported	3	7
Parametrial involvement	Negative	25	80.6
	Positive	6	19.4
Surgical border invasion	Negative	30	96.8
	Positive	1	3.2
Lymphovascular invasion	Negative	16	51.6
	Positive	11	35.5
	Not reported	4	12.9

Adnexal metastasis	Negative	25	80.6
	Positive	1	3.2
	Not reported	5	16.1
Depth of the stromal invasion	≤1/2	12	38.7
	>1/2	16	51.6
	Not reported	3	9.7
Endometrial/uterine invasion	Negative	24	77.4
	Positive	4	12.9
	Not reported	3	9.7
Adjuvant radiotherapy	Not received	7	22.6
	Received	24	77.4
Type of adjuvant radiotherapy	Only radiotherapy	2	6.8
	Concomitant chemoradiotherapy	19	61.3
	Not reported	3	9.7

[1] Lymph node metastasis evaluated in 38 patients (31 patients underwent radical hysterectomy+lymphadenectomy and seven patients underwent extraperitoneal/transperitoneal lymphadenectomy+radiotherapy), SD: Standard deviation, NACT: Neoadjuvant chemotherapy, FIGO: Federation of Gynecology and Obstetrics

Table 3. Factors predicting clinical response

Factors		SD+PD n (%)	CCR+PCR n (%)	p
Age[1]	≤48 years	16 (72.2)	6 (27.3)	0.845
	>48 years	14 (70)	6 (30)	
FIGO 2009 stage	I	18 (64.3)	10 (35.7)	0.119
	II	13 (86.7)	2 (13.3)	
Tumor size before NACT[1]	≤50 mm	19 (79.2)	5 (20.8)	0.245
	>50 mm	12 (63.2)	7 (36.8)	
NACT combination	CF	27 (75)	9 (25)	0.335
	Others[2]	4 (57.1)	3 (42.9)	
Number of NACT cycles	2	14 (87.5)	2 (12.5)	0.083
	3	17 (63)	10 (37)	
Histopathologic type	Squamous cell	28 (71.8)	11 (28.2)	0.892
	Others[3]	3 (75)	1 (25)	

[1]Median value, [2]Carboplatin and paclitaxel, cisplatin and UFT™, [3]Adenocancer+adenosquamous cell cancer, CCR: Complete clinical response, PCR: Partial clinical response, SD: Stabile disease, PD: Progressive disease, NACT: Neoadjuvant chemotherapy, CF: Cisplatin and 5-fluorouracil, FIGO: Federation of Gynecology and Obstetrics

stage CC. After NACT, these studies have reported that the CCR rates ranged from 0% to 50%, whereas CCR + PCR rates ranged from 45% to 95%[12,15-46]. In the survival analysis, the 5-year OS and DFS rates varied between 28% and 92.1% and from 29% to 85%, respectively[12,15,19,20,24-26,28,30,34,35,39-42,44-52]. The results of the present study were analyzed in the light of these literature data.

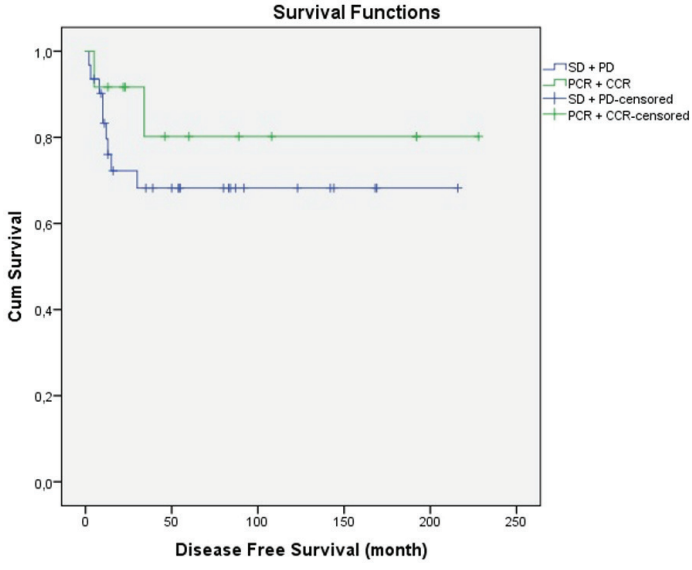

Figure 1. Disease-free survival and clinical response

CCR: Complete clinical response, PCR: Partial clinical response, SD: Stable disease, PD: Progressive disease

Discussion

NACT is the standard treatment in especially breast and head-neck cancers and in many other solid tumors. Despite years of experience, the value of NACT in the treatment of CC is still undetermined.

In theory, NACT is expected to increase the operability by decreasing the tumor size and to improve the surgical prognostic factors by destroying micrometastasis. CT given before the RH and RT, which damage the circulation of the tissues, is thought

Table 4. Effects of clinical and pathological parameters on disease-free survival

Clinical and pathological parameters		5-year disease-free survival (%)	p
Age[1]	≤48 years	86	0.141
	>48 years	61	
FIGO 2009 stage	I	73	0.710
	II	72	
Histopathologic type	Squamous cell cancer	72	0.866
	Others2	67	
NACT protocol	Cisplatin and 5-fluorouracil	76	0.233
	Others3	57	
Treatment after NACT	Surgery	72	0.841
	Radiotherapy	74	
Rate of decrease in tumor size after NACT	Did not decreased	86	0.304
	<25%	50	
	≥25%, <50%	70	
	≥50% (with gross tumor)	73	
	No gross tumor (clinically)	100	
Clinical response	SD+PD	68	0.374
	CCR+PCR	80	
Number of courses	2	67	0.530
	3	74	
Tumor size before NACT (mm)[1]	≤50	77	0.396
	>50	66	
Tumor size after NACT (mm)[1]	≤30	78	0.238
	>30	64	
Lymph node metastasis[4]	Negative	67	0.326
	Positive	81	

[1]Median value, [2]Adenocancer+adenosquamous cell cancer, [3]Carboplatin/paclitaxel, cisplatin/UFT™
[4]Lymph node metastasis evaluated in 38 patients (31 patients underwent radical hysterectomy+lymphadenectomy and seven patients underwent extraperitoneal/transperitoneal lymphadenectomy+radiotherapy), NACT: Neoadjuvant chemotherapy, SD: Stable disease, PD: Progressive disease, CCR: Complete clinical response, PCR: Partial clinical response, FIGO: Federation of Gynecology and Obstetrics

to be having more robust antitumoral effects.

Some of the studies have supported this theoretical approach. These studies, which were generally phase II studies, have shown that NACT improved surgical prognostic factors[19,47,49,53]. However, recent reports that compare the NACT followed by RH and RH alone do not show this improvement[12,19,40,46-49,54].

Studies have reported that CCR obtained by NACT ranged from 0% to 50% (OCR, 25-95%) (Table 5). After NACT, 28-100% of the patients became eligible for surgery[16-21,28,29,31,33,40,42,46,48,49,55]. One of the reasons of the variability of these rates is the non-homogeneity of the stages analyzed in the studies. In most of these studies, patients had locally advanced CC ranging from stage IB2 to IVA. However, the response after NACT is directly correlated to the disease stage. In a meta-analysis, Eddy et al.[15] reported that the CCR of 28% in stage IB2-IIA decreases to 7% in stage IV. Similar results were reported in other studies[23,25,30,43,50].

The operability rates change parallel to the clinical response, and stage is also a determining factor. Dueñas-Gonzales et al.[30] showed that operability is 83% in stage IB2, 60% in stage IIB, and 40% in stage IIIB. Gadduci et al.[43] also reported that operability decreases in the advanced stage. These studies have revealed that NACT is inappropriate for CC in advanced stage because of the high probability of RT need, which should be limited to early disease stages.

In the study by Li et al.[46], the DFS [hazard ratio (HR) 0.4, 95% confidence interval (CI) 0.1-08%; p=0.027] and OS (HR: 0.1, 95% CI: 0.01-0.8; p=0.026) rates were better in complete clinical responders than in non-responders. In the present study, the 5-year DFS rate was 80% in responders and 68% in non-responders. Similar results are demonstrated in other studies[8,56]. By contrast, Pat CR determines the survival, which varied from 0% to 26%[12,16,17,19,20,23,25,34,36,46,55].

Similar to response and operability, reported survival rates are varied (Table 6). The 5-year DFS and OS rates varied between 29% and 85% and 28% and 92.1%, respectively[12,19,25,40,42,46,48,51,52]. Our results are within this wide range (5-year DFS, 72%; 5-year OS, 87%). Lymph node metastasis, disease stage, parametrial involvement, stromal invasion, surgical border positivity, lymphovascular space invasion, histologic type, Pat CR, and tumor size before and after treatment carry prognostic significance for survival. An et al.[57] reported that deep stromal invasion, lymph node metastasis, and tumor size after NACT affect the OS rate ($p<0.05$). The authors also showed that stromal invasion was an independent risk factor of DFS rate ($p<0.05$), and the OS rate was significantly affected by tumor size >3 cm after NACT in a multivariate analysis. In the present study, none of risk factors were significant in the survival analysis.

Understanding the place of the NACT is difficult owing to the variability of the reported results. The non-homogeneity of the study group in terms of stages is one of the reasons of the variability. Survival after NACT is lower in advanced stages[25,58-60], and NACT has not any contribution to survival in these stages[30]. Results of the studies in which study group consisted of patients with only stage IB2 disease were very variable (Table 6)[12,15,19]. One of the reasons is the uncertainty of the clinical staging.

Table 5. Clinical response rates after NACT reported in studies

Author	Stage	Neoadjuvant chemotherapy protocol	Interval (day)	CCR (%)	PCR (%)	SD (%)	PD (%)
Eddy et al., 1995[15]	IB2	Cis+vinc, 3 cycles	10 d	6	76	15	3
Lacava et al., 1997[16]	IIB-IVA	Vineralbine, 12 doses	7 d	5	40	38	17
Giardina et al., 1997[17]	IB2-IIIB	Cis, 4 doses	7 d	28	57	15	
Fujiwaki et al., 1997[18]	IIB	Cis+peplo or doxo, 1 cycle	-	4	75	21	
Serur et al., 1997[19]	IB2	Cis+MTX+bleo, 3 cycles	21 d	10	80	10	
		Cis+vinc+bleo, 3 cycles	10 d				
Zanetta et al., 1998[20]	IB2-IVA	Cis+ifos+pacli, 3 cycles	21 d	28.9	55.3	13.2	2.6
Sugiyama et al., 1999[21]	IIIB	Cis or carb+peplo, 2 cycles	21 d	7.1	60.7	32.1	0
Sugiyama et al., 1999[22]	IB2-IIIB	Cis+irinotecan, 2 or 3 cycles	28 d	13	65	17	4
Pignata et al., 1999[23]	IB2-IVB	Cis+vineralbine, 3 cycles	21 d	22	42	18	18
Chang et al., 2000[24]	IB2-IIA	Cis+vinc+bleo, 3 cycles	10 d	23.5	55.8	13.2	4.4
Etcheverry et al., 2000[25]	IB2-IIIB	Cis+ifos+5-FU, 3 cycles	21 d	30	55	10.6	4.4
Hwang et al., 2001[26]	IB2-IIB	Cis+vinc+bleo, 3 cycles	21 d	50	43.7	6.3	0
Aoki et al., 2001[27]	IB2-IIB	Cis+vinc+peplo, 2 cycles	21 d	0	86	14	0
Aoki et al., 2001[28]	IB-III	Cis(IA)+5-FU(IA), 2 or 3 cycles	21 d	0	64	27	9
Porzio et al., 2001[29]	IB2-IIB	Cis+vinc+bleo, 3 doses	7 d	70		30	0
Dueños-Gonzales et al., 2001[30]	IB2-IIIB	Cis+gemci, 3 cycles	21 d	7.5	87.5	5	
D'Agostino et al., 2002[31]	IB2-IVA	Cis+pacli+epir, 2 or 3 cycles	21 d	19	59.5	12	9.5
Napolitano et al., 2002[32]	IB-IIIB	Cis+vinc+bleo, 3 cycles	21 d	22.6	56.6	20.8	
Dueños-Gonzales et al., 2003[33]	IB2-IIIB	Oxalip+gemci, 3 cycles	21 d	30	50	10	10
Dueños-Gonzales et al., 2003[34]	IB2-IIIB	Carb+pacli, 3 cycles	21 d	9	86	5	
Huang et al., 2003[35]	IB2-IIA	Cis+vinc+bleo, 3 cycles	10 d	18.1	61.3	20.6	0
Termrungruanglert et al., 2005[36]	IB2	Cis+gemci, 2 cycles	21 d	33.3	55.5	11.1	0
Fuso et al., 2005[37]	IB2-IIB	Cis-based CT*, 3 cycles	21 d	24.7	39.7	35.6	
Choi et al., 2006[38]	IB1-IIA	Cis+5-FU, 2 or 3 cycles	21 d	16	50	34	0
Eddy et al., 2007[12]	IB2	Cis+vinc, 3 cycles	10 d	15	37	45.5	2.3
Gong et al., 2012[39]	IB2-IIB	Cis-based protocols, 1 or 3 cycles	Change*	4	86	10	
Katsumata et al., 2013[40]	IB2-IIB	Cis+mit+bleo, 2 or 4 cycles	21d	66		34	
Angioli et al., 2015[41]	IB2-IIB	Cis+pacli, 3 cycles	21 d	84.6		15.3	
Lee et al., 2016[42]	IB-IIB	Cis-based protocols (1 or 8 cycles)	Change*	84.6		15.3	
Gadducci et al., 2017[43]	IB-IVA	Cis+pacli, 6 cycles	7 d	35.2	47.1	17.7	0
Gadducci et al., 2018[44]	IB2-IIB	Cis-based protocols, 3 or 6 cycles	Change*	11	70.7	18.3	
Mori et al., 2019[45]	IB2-IIB	Irinotecan+nedaplatin, 2 cycles	21d	62.5		9.4	
Li et al., 2019[46]	IB2-IIB	Cis-based protocols	Change*	9	57	37	
Our study	IB2, IIA2, IIB	Cis-based protocols, 2 or 3 cycles	Change*	9.3	18.6	69.9	2.3

* Interval changes according to protocols, CCR: Complete clinical response, PCR: Partial clinical response, SD: Stable disease, PR: Progressive disease, Cis: Cisplatinum, Vinc: Vincristine, Peplo: Peplomycin, Doxo: Doxorubicin: Ifos: Ifosfamide, Bleo: Bleomycin, Pacli: Paclitaxel, MTX: Methotrexate, 5-FU: 5-fluorouracil, Gemci: Gemcitabine, Epir: Epirubicin, Mit: Mitomisin, IA: Intra-arterial infusion

Table 6. Survivals rates obtained by neoadjuvant chemotherapy

Author	Stage	Neoadjuvant chemotherapy protocol	Interval (day)	Time for survival	DFS (%)	OS (%)
Eddy et al., 1995[15]	IB2	Cis+vinc, 3 cycles	10 d	2 years	85	88
Kim et al., 1989[47]	IB2-IIIB	Cis+vinb+bleo, 3 cycles	21 d	2 years	94	94
Behtash et al., 2006[48]	IB2-IIA	Cis+vinc, 3 cycles	10 d	3 years	44	56
				5 Years	29	28
Eddy et al., 2007[12]	IB2	Cis+vinc, 3 cycles	10	3 years	59.7	NR
				5 years	56.2	NR
Etcheverry et al., 2000[25]	IB2-IIIB	Cis+ifos+5-FU, 3 cycles	21 d	5 years	78	78
Serur et al., 1997[19]	IB2	Cis+MTX+bleo, 3 cycles	21 d	5 years	80	80
		Cis+vinc+bleo, 3 cycles	10 d			
Sardi et al., 1997[49]	IB1-IB2	Cis+vinc+bleo, 3 cycles	10 d	7 years	88	82
Hwang et al., 2001[26]	IB2-IIB	Cis+vinc+bleo, 3 cycles	21 d	10 years	80	97.5
Zanetta et al., 1998[20]	IB2-IVA	Cis+ifos+pacli, 3 cycles	21 d	Median f-u:16 months	76	94
Duenas-Gonzales et al., 2003[34]	IB2-IIIB	Carb+pacli, 3 cycles	21 d	Median f-u:21 months	79	79
Duenas-Gonzales et al., 2001[30]	IB2-IIIB	Cis+gemci, 3 cycles	21 d	Median f-u:28 months	55	62
Aoki et al., 2001[28]	IB-III	Cis+5-FU (IA), 2 or 3 cycles	21 d	Median f-u:30 months	18.2	27.3
Chang et al., 2000[24]	IB2-IIA	Cis+vinc+bleo, 3 cycles	10 d	Median f-u:39 months	69	79
Buda et al., 2005[50]	IB2-IVA	Cis+ifos+pacli, 3 cycles	21 d	Median f-u:43 months	74	75
		Cis+ifos, 3 cycles			70	63
Huang et al., 2003[35]	IB2-IIA	Cis+vinc+bleo, 3 cycles	10 d	Median f-u:49 months	65	69
Yin et al., 2011[51]	IB2-IIB	Cis-based protocols, 2 or 3 cycles	Change*	5 years	85	88.7
Gong et al., 2012[39]	IB2-IIB	Cis-based protocols, 1 or 3 cycles	Change*	2 years	93	95.5
Katsumata et al., 2013[40]	IB2-IIB	Cis+mit+bleo, 2 or 4 cyles	21 d	5 years	59.9	70
Angioli et al., 2015[41]	IB2-IIB	Cis+pacli, 3 cycles	21 d	4 years	80	84
Lee et al., 2016[42]	IB-IIB	Cis-based protocols (1 or 8 cycles)	Change*	5 years	75.6	92.1
Gupta et al., 2018[52]	IB2-IIB	Cis+pacli, 3 cycles	21 d	5 years	69.3	75.4
Gadducci et al., 2018[44]	IB2-IIB	Cis-based protocols, 3 or 6 cycles	Change*	Median f-u:89 months	72	77
Mori et al., 2019[45]	IB2-IIB	Irinotecan+nedaplatin, 2 cycles	21 d	5 years	78.8	89.7
Li et al., 2019[46]	IB2-IIB	Cis-based protocols	Change*	5 years	70	75
Our study	IB2, IIA2, IIB	Cis-based protocols, 2 or 3 cycles	Change*	5 years	72	87

*Interval changes according to protocols, DFS: Disease-free survival, OS: Overall survival, NR: Not reported, median f-u, median follow-up, Cis: Cisplatinum, Vinc: Vincristine, Vinb: Vinblastine, Ifos: Ifosfamide, Bleo: Bleomycin, Pacli: Paclitaxel, MTX: Methotrexate, 5-FU: 5-fluorouracil, Gemci: Gemcitabine, IA: Intra-arterial

The diversity of the CT protocols may be another reason for the variability of the results. CT protocols do not affect response and survival because many of them are cisplatin-based[55]. A multicenter randomized phase III trial in Italy comparing cisplatin/ifosfamide/paclitaxel combination with cisplatin/ifosfamide showed that triple NACT protocol improved the CCR significantly (20% and 9%)[50]. No difference was found between the two C protocols in terms of the operability and survival. In a randomized controlled study by Yang et al.[61], NACT combination irinotecan plus cisplatin (IP group) and paclitaxel plus cisplatin (TP group) were compared. The authors reported no difference between the two groups in terms of OS and DFS (OS, p=0.212; DFS, p=0.296).

Data related to the CF combination are generally derived from concurrent chemoradiotherapy. The reported Pat CR changed from 40% to 67.5%[62-64]. However, there is a limited number of

studies that have used this NACT. Choi et al.[38] reported that the CCR was 16% in stage IB1-IIA, the 5-year OS rate was 80.7%, and the 10-year OS was 77%. Etcheverry et al.[25] reported CCR and Pat CR of 30% and 13%, respectively (stage IB2-IIIB), in which ifosfamide was added to the CF combination. The 5-year OS was 78% in this study.

In a meta-analysis, Zhu et al.[65] included 4727 patients, in which the patients had FIGO stage IB and IIB CC and NACT combination consisted of platinum and/or taxane-based CT. Their clinical response rate ranged from 58.49% to 86.54%, and the pathological response rate ranged from 7.5% to 78.81%. Moreover, Zhu et al.[65] indicated that clinical and pathologic responses were associated with a favorable prognosis. Meng et al.[66] compared NACT+RH with RH. As NACT combination, cisplatin plus paclitaxel were implemented. The clinical response rate (CCR+PR) was 80.5% in the RH group and 91.2% in the NACT+RH group (p=0.048). In our study, which includes stage IB2-IIB, the CCR was 9.3%, the Pat CR was 6.9%, and the 5-year OS rate was 87%.

NACT for cervical cancer meta-analysis collaboration reevaluated the data of 21 phase III trials performed between 1975 and 2000 and reported them in a meta-analysis[11]. Results are divided into the two groups. Studies that compared NACT followed by RT (NACT+RT) and RT alone (16 studies, n=2.074) were included in the first group, and studies that compared NACT+RH and RH (5 studies, n=872) were included in the second group. After the assessment of the second group, NACT + RH decreased the mortality rate by 35% and improved the survival by 12% when compared with the RT group. Only two of the five studies in the second group included stage IB2 tumors[24,55]. Benedetti-Panici et al. [55] defined the survival advantage by NACT in stage IB2 disease in the subgroup analysis, but Chang et al.[24] did not show any advantage. In addition, Chang et al.[24] showed that clinical response was higher in the RT group, but Sardi et al.[49] showed that the clinical response was better in the NACT group than in NACT+RH+adjuvant RT and RH+adjuvant RT groups. Recently, Zou et al.[67] published a meta-analysis involving 2.270 patients with stage IB2-IIB CC and evaluating the efficacy of concurrent chemoradiation and NACT followed by radical surgery (NACT+RH). They stated that compared with the concurrent chemoradiation group, the NACT+RH group did not have a survival advantage (OS, p=0.07; DFS, p=0.82)[67]. Patients receiving NACT with concurrent chemoradiation were compared in another randomized phase II study, and authors revealed that prognosis in the concurrent chemoradiation group was more favorable than that in the NACT group (DFS, HR 1.84, 95% CI 1.04-3.26, p=0.033; OS, HR 2.79, 95% CI 1.29-6.01, p=0.006)[68]. European Organisation for Research and Treatment of Cancer (ClinicalTrials.gov identifier: NCT00039338) investigated the effect of NACT+RH against concurrent chemoradiation in patients with stage IB2-IIB disease using cisplatin-based CT regimens. Unfortunately, some of its data are still not yet published. The results of this study will shed light on the management of these patient groups.

Aoki et al.[27] compared NACT+RH and RH alone (stage IB-IIB) and reported that the pathological prognostic factors and survival were better in the NACT group. Similar results were also reported by Namkoong et al.[69] (stage IB-IIB). By contrast, in their randomized controlled trial, Yang et al.[61] showed that pathologic prognostic factors were improving in the NACT group, but it does not affect the survival. Yang et al.[54] found similar survival results between the NACT+RH and RH groups in their meta-analysis of 16 studies. A retrospective study[48] compared NACT+RH and RH alone in early-stage CC, and a prospective phase III study by GOG[12] was published. These studies concluded that NACT has no place in the treatment of early-stage CC. In the study of GOG, surgical prognostic factors and survival in stage IB2 tumors were not improved by NACT (cisplatin/vincristine, every 10 days, three courses). The 5-year OS was 56.2% in the NACT group and 53.8% in the RH group[12].

An article compared the effectiveness of NACT or primary RH in patients with stage 1B2 CC previously treated in our clinic[70]. In this study, 24 patients who received NACT followed by radical surgery were compared with 15 patients who underwent primary radical surgery. Patients were divided into three groups, including RH alone, NACT unresponder group, and NACT responder group. No difference was found between these groups in terms of recurrence, DFS, and OS.

Study Limitations

The retrospective design is the most critical limitation of the present study. Moreover, improvements in surgical and adjuvant therapy modalities over years may affect the results. In addition, the small sample size and the fact that NACT was not compared with other treatment methods (RH, CCRT, etc.) also limited the interpretation of the results. As strengths, detailed clinical-pathological characteristics and adjuvant treatments of the patients were evaluated. Pathologic examinations were performed by experienced gynecological pathologists. Follow-up periods of the patients were long. Additionally, the results were revised in the light of various relevant studies published in the literature.

Conclusion

The value of NACT in the treatment of CC is still being debated and discussed. At present, it is thought that NACT may be used in locally advanced CC, but results reveal that this is not feasible. By contrast, we think that new drugs, new combinations, and new protocols of NACT could achieve successful treatment of CC, as in theory.

Ethics

Ethics Committee Approval: This study was approved by the Etlik Zübeyde Hanım Women's Health Training and Research Hospital Ethical Committee (file no. 90057706-799/08; 05.06.2020).

Informed Consent: Medical records of patients with stage IB2, IIA2, or IIB CC between 1998 and 2020 were reviewed retrospectively.

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Authorship Contributions

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Does polycystic ovary syndrome with phenotype D affect the cardiovascular endurance, core endurance, body awareness, and the quality of life? A prospective, controlled study

Fenotip D'li polikistik over sendromu kardiyovasküler enduransı, kor enduransı, vücut farkındalığını ve yaşam kalitesini etkiler mi? Prospektif, kontrollü çalışma

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Abstract

Objective: This study evaluates cardiovascular endurance, core endurance, body awareness, and the quality of life in normal-weight women with polycystic ovary syndrome.

Materials and Methods: This study included a total of 101 normal-weight women (51 with and 50 without polycystic ovary syndrome). Cardiovascular endurance was evaluated with the 20-meter Shuttle Run test, and maximum oxygen consumption was calculated. Core endurance was evaluated with core stability tests, body awareness with the body awareness questionnaire, and the quality of life with short form-36. Blood lipids, glucose, insulin, homeostatic model assessment for insulin resistance (HOMA-IR), hormonal profile, and high-density and low-density lipoprotein cholesterols were measured.

Results: Maximum oxygen consumption, core endurance, body awareness questionnaire, and short form-36 results were lower in women with polycystic ovary syndrome than healthy women ($p<0.05$). There was a significant correlation between core endurance tests, high-density lipoprotein cholesterol, maximum oxygen consumption, and homeostatic model assessment for insulin resistance scores ($p<0.05$).

Conclusion: When normal-weight women with polycystic ovary syndrome and control groups with similar androgen levels and body mass index profiles were compared, women with polycystic ovary syndrome had lower aerobic capacity and muscle endurance. This suggests that the adverse metabolic profile of polycystic ovary syndrome can limit physical function.

Keywords: Polycystic ovary syndrome, exercise tolerance, core stability, body image

Öz

Amaç: Bu çalışmada, normal kilolu polikistik over sendromlu kadınların kardiyovasküler enduransının, kor enduransının, vücut farkındalığının ve yaşam kalitesinin değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu çalışmaya toplam 101 normal kilolu kadın (51 polikistik over sendromlu ve 50 polikistik over sendromsuz) dahil edildi. Kardiyovasküler endurans, 20 metrelik Shuttle Run testi ile değerlendirildi ve maksimum oksijen tüketimi hesaplandı. Kor enduransları kor stabilite testleri ile, vücut farkındalığı vücut farkındalık anketi ile, yaşam kaliteleri kısa form-36 ile değerlendirildi. Kan lipidleri, glukoz, insülin, homeostatik model değerlendirmesi (HOMA-IR), hormon profilleri ve yüksek yoğunluklu ve düşük yoğunluklu lipoprotein kolosterol ölçüldü.

Bulgular: Polikistik over sendromlu kadınlarda maksimum oksijen tüketimi, kor endurans, vücut farkındalık anketi ve kısa form-36 sonuçları sağlıklı kadınlara göre daha düşüktü ($p<0,05$). Kor endurans testleri ile yüksek yoğunlujlu, lipoprotein kolesterol, maksimum oksijen tüketimi ve homeostatik model değerlendirmesi insülin direnci skorları arasında anlamlı bir ilişki vardı ($p<0,05$).

PRECIS: We evaluated cardiovascular endurance (CE), core endurance, body awareness, and the quality of life in normal-weight women with phenotype D-polycystic ovary syndrome (PCOS) and healthy women.

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Sonuç: Polikistik over sendromlu normal kilolu kadınlar ile androjen düzeyleri ve vücut kütle indeks profilleri benzer olan kontrol grupları karşılaştırıldığında, polikistik over sendromlu kadınların aerobik kapasiteleri ve kas enduransları daha düşüktü. Bu, polikistik over sendromun olumsuz metabolik profilinin fiziksel işlevi sınırlayabileceğini düşündürmektedir.
Anahtar Kelimeler: Polikistik over sendromu, egzersiz toleransı, kor stabilite, beden imajı

Introduction

Polycystic ovary syndrome (PCOS) affects 6-10% of reproductive-aged women. PCOS is a common endocrinological disease that can have progressive metabolic effects[1]. Hyperandrogenism, ovulatory dysfunction, dyslipidemia, and insulin resistance (IR) are the principal features of this syndrome[2]. Also, women with PCOS have more cardiovascular risk factors, such as dyslipidemia, hypertension, glucose intolerance, and diabetes, compared with women without PCOS[2-4].

The maximal oxygen consumption (VO2max) is the highest amount of oxygen used by the body during maximal exercise, and the risk of cardiovascular disease increases when VO2max is reduced. Studies are contradictory about whether PCOS affects VO2max. One study[4] stated that VO2max values of women with and without PCOS were similar. A second study[4] found that VO2max values of women with PCOS were lower than those without PCOS.

In addition to the change in V02max, muscle function may also be affected by biochemical results in PCOS. Insulin is the primary regulator of muscle proteins. It can stimulate mitochondrial protein synthesis[2]. Androgens can increase muscle strength or endurance and may promote free-fat muscle growth. When the amount of high-density lipoprotein cholesterol (HDL) decreases, it can decrease muscular function by increasing the release of proinflammatory cytokines[5-7]. Core endurance is a muscle function (abdominal, paraspinal, gluteal, diaphragm, and pelvic floor muscles) PCOS can affect core muscles due to impaired biochemical profiles[8-10].

The endurance of the core muscles and the cardiorespiratory system are among the parameters of physical function. Body awareness informs the individual of the relationship between physical function and mental activity and explains how these factors affect the body. Alexander stated that the change in muscular functions decreases physical awareness and information from the body[8,9]. Based on studies showing changes in these factors[2-5,11,12], PCOS may also affect the body awareness. In addition, the change in the appearance of women with PCOS, infertility, decrease in performance, and cardiovascular disease risk may affect their quality of life (QOL) [13,14].

To the best of our knowledge, there are no studies comparing women with and without PCOS regarding VO2max, core endurance, QOL, and body awareness[5]. Studies on PCOS have mainly been conducted on obese women[4,8,11,12-18]. We wanted to exclude the obesity factor to examine the effect of PCOS. The present study evaluates VO2max, core endurance, body awareness, and QOL in normal-weight women with and without PCOS and investigate the effect of PCOS on these parameters.

Materials and Methods

Study Design and Participants

This case-control study was conducted prospectively in the gynecology department of a tertiary hospital. Also, permission was granted from the Clinical Research Ethics Committee of the University before the study (decision no: 2017-KAEK-189_2020.01.08_04). All participants were informed about the study based on the 1975 Helsinki Declaration. All participants also signed informed consent that they agreed to participate in the study.

Inclusion criteria were weight stability (<2.0 kg weight changes) for the last 3 months, 18-40 years of age, willingness to participate in the study, and having normal weight [body mass index (BMI): 18.5-24.99 kg/m^2]. The exclusion criteria for both groups included smoking, volunteers who performed regular exercise, cardiovascular and chronic diseases, androgen-secreting tumors, late-onset 21-hydroxylase deficiency, drugs (such as hormones, anti-diabetic agents, and oral contraceptives), and pregnancy. Participants with a history of angina or any other cardiopulmonary or physical symptoms that could affect exercise performance were also excluded[12].

The diagnosis of PCOS was made according to the Rotterdam criteria[19]: those with two of the three criteria were diagnosed as PCOS: oligo and/or anovulation (>35 days or <8 spontaneous menstruation/year), biochemical and/or clinical (Ferriman-Gallwey score >8) hyperandrogenism, and polycystic ovary (12 or more follicles 2-9 mm in diameter in each ovary and/or ovarian volume >10 mL). We included only phenotype D-PCOS women in our study, as hyperandrogenism may affect muscle strength. In our study, the women in the PCOS group were identified according to the specific European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine phenotypes as type D: oligo and/or anovulation and polycystic ovary[20]. Patients who applied for routine gynecological examination without oligomenorrhea and did not meet the PCOS diagnostic criteria were included in the control group[5].

According to the reference study results, they had a large effect size (d=0.71)[5]. Assuming we could achieve a lower effect size level (d=0.5), a power analysis was performed before the study. Accordingly, when at least 100 participants 50 from the PCOS group and 50 from the control group) were included in the study, which would result in 80% power with a 95% confidence level (5% type 1 error rate).

Evaluations

Demographic characteristics [age (years), education (primary school, high school, or university), and employment status (yes or no)] were questioned, and waist-hip ratio (WHR) was calculated.

Ultrasonography: Morphological features of the ovaries of all participants were examined by transabdominal/transvaginal ultrasonography (GE Voluson E8, USA)[4,12].

Biochemical analysis: Blood samples were collected on the second or third days of the menstrual cycle. Serum insulin, luteinizing hormone, follicle-stimulating hormone, and total testosterone levels were measured via electrochemiluminescence immunoassay on a Roche COBAS 6000 e601 (Roche Diagnostics, Mannheim, Germany) autoanalyzer. Fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were analyzed on a Roche COBAS 6000 c501 (Roche Diagnostics) autoanalyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula when the TG level was less than 400 mg/dL. Homeostatic model assessment for insulin resistance (HOMA-IR) was used to calculate IR. HOMA-IR (fasting blood glucose mg/dL x fasting insulin mIU/L/405) value ≥2.5 was accepted as the presence of IR[3,4,19].

Cardiorespiratory endurance: The cardiovascular endurance level (VO2max) of all participants was evaluated with the 20-meter shuttle run test (20mSRT)[8,21]. This test was developed in accordance with the Eurofit test Battery (1988) directives. In the 20mSRT, the participants ran continuously on a 20-m-track at an initial speed of 8 km/h, which increased 0.5 km/h per one minute. The running pace was adjusted using a timer and signal generator. The women who took the test were asked to complete the 20-m-course at each signal. The test was terminated for women unable to reach the lines one meter before the lines that determined the 20 meters when the signal was received. The running pace in accordance with the test protocol was provided by the signals obtained from the Pro Tmr Esc 1000 sport test timer[21,22]. VO2max levels in mL/kg/min were calculated using Leger's formula[23]:

VO2 peak=31.025+3.238*S - 3.248*A + 0.1536*S*A

S=final speed (kmh-1); A=age (years).

Core endurance: A protocol developed by McGill was used to assess the endurance of the core muscles. Core endurance was evaluated with a core stability test (trunk flexion, extension, and lateral right/left bridge tests). In the trunk flexion test (TFT), the participants were seated on the treatment table with a wedge that provided a 45° flexion on their back. The knees were brought to 90° flexion. The test was ended when the upper body could not maintain the 45° angle (Figure 1a). In the trunk extensor endurance test (TEET), the participants were asked to lie on the treatment table in the prone position. In the prone position, the spina iliaca anterior superior part of the participant was placed on the edge of the table. The body was suspended from the table (Figure 1b). The lateral bridge test (RBT/LBT): Participants were asked to build a side bridge by standing on the forearms with the elbow flexed at 90° in the side-lying position. They were also instructed to lift the hip off the table with the other arm and hand. The test was terminated when the straight body position could not be maintained. This test was repeated for both the right and left sides separately. The time to maintain these positions was recorded (Figure 1c)[10,24,25].

Body awareness: Participants' body awareness was evaluated using the body awareness questionnaire (BAQ) developed by Shields et al.[26] Turkish validity and reliability of the questionnaire were carried out by Karaca and Bayar[27] Participants' body awareness was evaluated using the BAQ[13]. BAQ aims to evaluate cases with sensitivity to body cycles and rhythms, the ability to perceive minor changes in normal functioning, and the ability to predict bodily responses. BAQ consists of 18 items and 4 subdimensions. The four subdimensions are as follows: body reactions estimation (BAQ-I), sleep-wake cycle (BAQ-II), prediction at the onset of the disease (BAQ-III), and paying attention to the changes in body processes and reactions (BAQ-IV). Each of the 18 expressions is scored between 1 and 7 (1=Not at all true about me, 7=Very true about me)[26]. The higher the score obtained from the questionnaire, the higher the level of body awareness.

QOL: Participants' QOL was evaluated using the short form-36 (SF-36) scale[28]. Turkish validity and reliability of the scale were conducted by Koçyiğit et al.[28] in 1994. The SF-36 consists of 36 items and eight subdimensions. Physical function, role restriction due to physical problems, role restriction due to emotional problems, mental health perception, social function, general health perception, body pain, and vitality comprised the subdimensions. The scoring of each section was between 0 and 100. Zero indicated the lowest QOL, whereas 100 indicated the highest QOL[14].

Figure 1a. Trunk flexor bridge test, **b.** Trunk extensor bridge test, **c.** Left/right side bridge test

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 software. Continuous variables were defined by the mean ± standard deviation, median (minimum-maximum values), and categorical variables were defined by number and percent. The Kolmogorov-Smirnov test was used for the determination of normal distribution. For independent group comparisons, an independent samples t-test was used when parametric test assumptions were provided. The Mann-Whitney U test was used when parametric test assumptions were not provided. A Spearman correlation analysis was performed to analyze the relationships between continuous variables. The effect of PCOS on continuous variables was determined by linear regression analysis models using dummy variables. Statistical significance was determined as $p<0.05$.

Results

In the present study, 101 women [control group (50 women) and PCOS group (51 women)] were included in the study. Their demographic information is provided in Table 1. There was no significant difference in women's age, education, BMI, and occupation status between the groups ($p>0.05$).

The comparison of the biochemical results, VO2max, core endurance tests, BAQ, and the SF-36 scores are shown in Table 2. WHR and HOMA-IR index variables were higher in the PCOS group compared with the control group ($p<0.05$). HDL-C, VO2max, TFT, TEET, RBT and LBT, BAQ-total, BAQ-I, BAQ-II, BAQ-IV, physical function, physical role limitations, vitality, mental, pain, and general health scores of the PCOS group were lower compared with those of the control group ($p<0.05$).

The correlations of the biochemical results, VO2max, and core endurance are shown in Table 3. A positive correlation

Table 1. Demographic characteristics of participants

	Control (n=50)	PCOS (n=51)	p
Age (y)*	25 (18-34)	24 (18-38)	0.27 (z=-1.10)
BMI (kg/m^2)***	22.7±1.33	23.0±1.12	0.317 (t=3.568)
			Total
Education status** 0.88 (χ^2 0.24)			
Primary education	1 (2%)	1 (2%)	2 (2%)
High school	19 (38%)	17 (33.3%)	36 (35.6%)
University	30 (60%)	33 (63.7%)	63 (62.4%)
Occupation 0.622 (χ^2 0.24)**			
Worker	25 (50%)	23 (45.1%)	48 (47.5%)
Housewife	25 (50%)	28 (54.9%)	53 (52.5%)

BMI: Body mass index, y: Years, M: Meter
Data is shown as median (min-max) and mean ± standard deviation
*Mann-Whitney U test (z), **chi square test (χ^2), ***Independent samples t-test (t)

Table 2. Biochemical analyses, core endurance tests, body awareness, and the quality of life of participants

	Control (n=50)	PCOS (n=51)	p
WHR	0.8 (0.64-1.07)	0.85 (0.69-1.37)	**0.0001*** (z=-4.748)
HDL-C (mg/dL)	61.55±15.43	49.17±9.37	**0.0001*** (t 4.882)
LDL-C (mg/dL)	90 (20.98-159)	88.42 (35-214.4)	0.555 (z=-0.591)
HOMA-IR (mg/dL ^{x} µU/mL)	1.79 (0.69-3.64)	2.81 (0.86-7.1)	**0.0001*** (z=-4.790)
Triglycerides (mmol/L)	67.45 (18.4-272)	75 (32.1-377)	0.055 (z=-1.922)
Total cholesterol (mmol/L)	148.45 (84.5-259)	153 (102.4-269)	0.257 (z=-1.134)
VO2max (mL/kg/minute)	24.7 (19-27.5)	23.2 (22-25.4)	**0.02*** (z=-2.332)
Total testosterone (ng/mL)	0.31 (0.1-0.62)	0.32 (0.12-0.69)	0.691 (z=-0.398)
Core stability tests			
TFT (s)	42 (8-93)	22 (14-42)	**0.0001*** (z=-8.035)
TEET (s)	86 (40-120)	21 (10-60)	**0.0001*** (z=-8.513)
RBT (s)	37 (12-96)	17 (8-48)	**0.0001*** (z=-6.807)
LBT (s)	38 (17-153)	17 (7-30)	**0.0001*** (z=-8.358)
BAQ			
BAQ-total	90.5±11.12	73.12±4.42	**0.0001*** (t=10.360)
BAQ-I	37 (23-45)	28 (20-40)	**0.0001*** (z=-7.326)
BAQ-II	34 (26-42)	28 (24-37)	**0.0001*** (z=-6.233)
BAQ-III	16 (10-20)	16 (13-19)	0.056 (z=-1.915)
BAQ-IV	26 (14-33)	21 (16-26)	**0.0001*** (z=-5.197)
SF-36			
Physical function	87.5 (60-100)	75 (60-100)	**0.0001*** (z=-4.656)
Physical role difficulty	100 (0-100)	75 (50-100)	**0.0001*** (z=-5.895)
Emotional role difficulty	66 (0-100)	66 (0-100)	0.745 (z=-0.326)
Vitality	60 (30-85)	55 (40-80)	**0.001*** (z=-3.269)
Mental health	68.98±12.91	54.47±10.62	**0.0001*** (t=6.173)
Social function	75 (37.5-100)	75 (25-100)	0.136 (z=-1.491)
Pain	77.5 (50-100)	75 (45-100)	**0.0001*** (z=-3.502)
General health	67.5 (50-100)	60 (40-85)	**0.002*** (z=-3.061)

WHR: Waist-hip ratio, HOMA-IR: Homeostatic model assessment for insulin resistance, s: Second, SF: Short-form, BAQ: Body awareness questionnaire, BAQ-I (anticipation of bodily reactions); BAQ-II (sleep-wake cycle), BAQ-III (anticipation at the onset of the disease), and BAQ-IV (changes in body process), TFT: Trunk flexion test, TEET: Trunk extensor endurance test, LBT: Lateral left bridge test, RBT: Lateral right bridge test, mL: Milligram, dL: Deciliter, mL: Milliliter, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, PCOS: Polycystic ovary syndrome, Data is shown as median (minimum-maximum) and mean ± standard deviation, *Mann-Whitney U test (z), ***Independent Samples t-test (t)

Table 3. Correlations between VO2max, core stability, and biochemical analyses variables

		All patients			
		WHR	HDL-C (mg/dL)	HOMA-IR (mg/dL X µU/mL)	Triglyceride (mmol/L)
VO2max (mL/kg/minute)	r	-0.351	0.455	-0.444	-0.207
	p	**0.000**	**0.000**	**0.000**	**0.038**
TFT (s)	r	-0.459	0.525	-0.505	-0.277
	p	**0.000**	**0.000**	**0.000**	**0.005**
TEET (s)	r	-0.498	0.472	-0.425	-0.172
	p	**0.000**	**0.000**	**0.000**	0.086
RBT (s)	r	-0.480	0.462	-0.457	-0.192
	p	**0.000**	**0.000**	**0.000**	0.055
LBT (s)	r	-0.422	0.432	-0.489	-0.213
	p	**0.000**	**0.000**	**0.000**	**0.032**

WHR: Waist-hip ratio, HOMA-IR: Homeostatic model assessment for insulin resistance, s: Second, TFT: Trunk flexion test, TEET: Trunk extensor endurance test, LBT: Lateral left bridge test, RBT: Lateral right bridge test, mg: Milligram, dL: Deciliter, mL: Milliliter, HDL-C: High-density lipoprotein cholesterol, r: Spearman correlation

was found between HDL-C and VO2max, TFT, TEET, RBT, and LBT scores (p<0.001). There was a negative correlation between VO2max and HOMA-IR, WHR, and triglyceride values (p<0.05). A negative correlation was found between TFT and WHR, HOMA-IR, and triglyceride values (p<0.05). A negative correlation was found between TEET and WHR and HOMA-IR values (p<0.001). A negative correlation was found between RBT and WHR, LDL-C, HOMA-IR, and TC values (p<0.05).

Correlations of VO2max, core endurance, body awareness, and QOL scores are shown in Table 4. A moderate negative correlation was found between BAQ-III and VO2max (p<0.05). A weak positive correlation was found between TEET and vitality and BAQ-II results (p<0.05). A moderate positive correlation was found between mental function and VO2max (p<0.05).

Considering these differences, the factors that affected the PCOS factor were investigated. PCOS decreased HDL-C, HOMA-IR, TFT, TEET, RBT, LBT, BAQ-total, BAQ-I, BAQ-II, BAQ-IV, physical function, physical role limitation, vitality, mental, pain, and general score variables (standard beta values, from -0.910 to -276). Also, PCOS had an increasing effect on the HOMA-IR index and BAQ-III variables (Table 5).

Discussion

This study showed that normal-weight PCOS women with phenotype D had a lower cardiovascular endurance, core endurance, body awareness, and QOL than the women in the control group. To the best of our knowledge, this is the first study investigating the effect of normal weight in PCOS on these factors.

Many studies in the literature compare cardiovascular performance and QOL in PCOS[1-4,16]. However, these studies were primarily conducted on obese women[4,16,28]. In one of these studies[16], the mean BMI was 39.9±6.1 kg/m^2, while in another study, the mean BMI values were 34.1±5.5 in PCOS and 35.5±4.9 kg/m^2 in the control group[4]. In the present study, the BMI of women with and without PCOS were 23.4±0.97 and 22.6±1.10 kg/m^2, respectively. This study was the first study planned within normal BMI limits.

In one study[4], VO2max was evaluated, and its relationship with hormonal and metabolic factors was investigated. Another study[12] compared metabolic heart rate and VO2max factors in women with and without PCOS. In the present study, many factors that might affect the lifestyle in PCOS were investigated more comprehensively than studies reported in the literature.

In PCOS, proinflammatory cytokines can damage the endothelial tissue with the emergence of the inflammatory process. As a result of this tissue damage, the number of mitochondria and the amount of VO2max might decrease[1,3,4,17,28,29]. VO2max is the maximum amount of oxygen the body can use during activity and is known as the best indicator of cardiovascular endurance. Only a few small studies are evaluated VO2max values in women with PCOS[3,4]. Thomson et al.[4] found no difference in VO2max of women with and without PCOS who were overweight. Orio et al.[3] assessed VO2max in overweight women with PCOS and found that overweight women with PCOS had lower VO2max values than overweight women without PCOS[3,15]. In our study, normal-weight women with PCOS had lower VO2max capacities than healthy women. We also found that the PCOS factor affects HDL-C and HOMA-IR values a relationship between HDL-C and VO2max. This result might indicate that IR and HDL-C factors affected myocardial and skeletal muscle metabolism. So, HOMA-IR and HDL-C should be regularly examined in PCOS, even at a normal weight.

Table 4. Correlations between BAQ, SF-36, VO2max, and core stability test variables

VO2max		PCOS					Control				
		TFT	TEET	RBT	LBT	VO2max	TFT	TEET	RBT	LBT	
BAQ-total	r	-0.079	-0.131	-0.223	-0.035	-0.092	-0.084	-0.144	-0.009	0.032	-0.162
	p	0.581	0.359	0.115	0.808	0.520	0.563	0.317	0.949	0.828	0.260
BAQ-I	r	-0.074	-0.076	-0.158	0.016	-0.110	-0.151	-0.170	-0.030	-0.010	-0.123
	p	0.605	0.597	0.269	0.910	0.443	0.295	0.238	0.836	0.948	0.396
BAQ-II	r	0.268	0.241	**0.286***	-0.018	-0.223	-0.207	-0.262	-0.018	-0.063	-0.277
	p	0.057	0.089	**0.042**	0.902	0.117	0.149	0.066	0.900	0.661	0.051
BAQ-III	r	**-0.286***	-0.089	-0.015	-0.151	-0.078	0.113	-0.071	0.012	-0.118	-0.159
	p	**0.042**	0.535	0.916	0.289	0.584	0.436	0.623	0.933	0.413	0.269
BAQ-IV	r	-0.275	-0.098	-0.239	-0.195	-0.179	0.051	-0.027	0.013	0.114	-0.050
	p	0.051	0.494	0.091	0.170	0.210	0.724	0.853	0.929	0.432	0.729
Physical function	r	-0.006	-0.178	0.085	-0.169	-0.087	0.105	**-0.337***	0.100	0.239	**0.387****
	p	0.967	0.212	0.553	0.236	0.544	0.469	**0.017**	0.492	0.095	**0.006**
Physical role difficulty	r	0.111	-0.096	0.040	0.088	0.200	0.246	0.249	0.152	0.157	**0.324***
	p	0.438	0.504	0.778	0.540	0.160	0.084	0.081	0.293	0.276	**0.022**
Emotional role difficulty	r	-0.060	0.054	0.079	-0.052	0.088	-0.162	0.000	-0.204	0.018	-0.170
	p	0.674	0.705	0.583	0.716	0.539	0.262	1.000	0.155	0.904	0.237
Vitality	r	0.152	0.239	**0.387****	0.190	0.260	0.008	0.114	0.008	0.002	-0.037
	p	0.288	0.091	**0.005**	0.182	0.065	0.957	0.430	0.954	0.990	0.801
Mental health	r	**0.286***	0.270	-0.015	-0.166	-0.113	0.115	0.018	-0.091	0.232	0.198
	p	**0.042**	0.055	0.915	0.245	0.431	0.428	0.904	0.529	0.105	0.167
Social function	r	-0.008	0.112	0.003	-0.122	-0.101	0.098	-0.004	0.179	0.129	0.133
	p	0.956	0.432	0.981	0.394	0.479	0.497	0.980	0.213	0.374	0.355
Pain	r	0.254	0.177	0.214	0.098	0.237	-0.054	0.176	0.140	0.086	-0.048
	p	0.072	0.214	0.131	0.492	0.094	0.709	0.220	0.333	0.553	0.738
General health	r	0.218	0.037	0.226	0.273	0.058	0.018	-0.184	0.049	0.239	-0.030
	p	0.124	0.798	0.110	0.053	0.688	0.903	0.202	0.736	0.095	0.838

PCOS: Polycystic ovary syndrome, TFT: Trunk flexion test, TEET: Trunk extensor endurance test, LBT: Lateral left bridge test, RBT: Lateral right bridge test, BAQ: Body awareness questionnaire, BAQ-I (anticipation of bodily reactions); BAQ-II (sleep-wake cycle); BAQ-III (anticipation at the onset of the disease); and BAQ-IV (changes in body process), r: Spearman correlation

In addition to VO2max, muscle strength and endurance have also been shown to affect the risk of morbidity and mortality[8,9]. PCOS can alter muscle function through different metabolic and hormonal factors (such as hyperandrogenism, obesity, IR, HDL-C). Only two studies in the literature evaluated muscle strength in PCOS[4,5]. One study[4] found no difference in the muscle strength of women with and without PCOS who were overweight and ignored the effect of hyperandrogenism on muscle function. Another study[5] found that women with PCOS had higher muscle strength than the control group. They stated that hyperandrogenism is effective in muscle strength in the study and attributed it to androgen hormones. Based on these studies, we included women with phenotype D-PCOS. So, we excluded women with hyperandrogenism. Our study found that normal-weight women with PCOS (phenotype D) had lower muscle endurance than control group women. This may indicate that WHR and IR are more important than other factors in damaging muscle function by slowing protein synthesis.

Body awareness is related to the perception of physical, psychosomatic, and autonomic changes in the body[12,13,30]. Alexander stated that while working on body awareness,

Table 5. Effects of PCOS factor on biochemical parameters, VO2max, core endurance, BAQ, and SF-36 in the linear regression model

Dependent variable	Std. beta	t	p	95% CI Lower bound	95% CI Upper bound
HDL-C (mg/dL)	-0.441	-4.882	**0.0001***	-17.408	-7.347
LDL-C (mg/dL)	0.132	1.322	0.189	-4.345	21.714
HOMA-IR (mg/dL x µU/mL)	0.469	5.284	**0.0001***	0.730	1.608
Triglyceride (mmol/L)	0.161	1.620	0.108	-4.074	40.346
Total cholesterol (mmol/L)	0.097	0.968	0.335	-6.769	19.667
VO2max (mL/kg/minute)	-0.174	-1.760	0.082	-1.482	0.089
TFT (s)	-0.689	-9.471	**0.0001***	-32.745	-21.401
TEET (s)	-0.910	-21.777	**0.0001***	-65.090	-54.219
RBT (s)	-0.624	-7.954	**0.0001***	-29.033	-17.440
LBT (s)	-0.659	-8.725	**0.0001***	-36.803	-23.166
BAQ-total	-0.721	-10.360	**0.0001***	-20.711	-14.053
BAQ-I	-0.718	-10.261	**0.0001***	-9.985	-6.749
BAQ-II	-0.606	-7.576	**0.0001***	-6.553	-3.833
BAQ-III	0.219	2.229	**0.028***	0.109	1.881
BAQ-IV	-0.497	-5.704	**0.0001***	-5.491	-2.657
Physical function	-0.451	-5.029	**0.0001***	-13.981	-6.070
Physical role difficulty	-0.486	-5.539	**0.0001***	-27.758	-13.117
Emotional role difficulty	-0.011	-0.114	0.910	-14.421	12.855
Vitality	-0.276	-2.860	**0.005***	-9.691	-1.752
Mental health	-0.527	-6.173	**0.0001***	-19.173	-9.846
Social function	-0.146	-1.468	0.145	-13.916	2.083
Pain	-0.367	-3.930	**0.0001***	-18.015	-5.926
General health	-0.320	-3.361	**0.001***	-11.364	-2.927

Std. beta: Standardized coefficients beta, CI: Confidence interval; univariate linear regression analysis
TFT: Trunk flexion test, TEET: Trunk extensor endurance test, LBT: Lateral left bridge test, RBT: Lateral right bridge test, WHR: Waist-hip ratio, HOMA-IR: Homeostatic model assessment for insulin resistance, s: Second, cm: centimeter, BAQ: Body awareness questionnaire, SF: Short-form, BAQ-I (anticipation of bodily reactions); BAQ-II (sleep-wake cycle); BAQ-III (anticipation at the onset of the disease) and ^#BAQ-IV (changes in body process)

muscular changes can decrease the ability to receive information from the body[13]. To the best of our knowledge, there is no study in the literature evaluating body awareness in PCOS. In the present study, the body awareness of women with PCOS was lower than women without PCOS. VO2max and core endurance were also associated with body awareness parameters. These results may indicate that muscular and cardiovascular performance can be affected by changes in body awareness. This might suggest that providing body awareness training to women with PCOS in advanced studies might positively affect these parameters.

Body image deterioration and infertility can lead to self-confidence and psychological problems, reducing the QOL[26]. The number of studies examining the effects of PCOS on QOL and its subscales is limited. Most studies found lower physical role function, pain, vitality, social function, emotional function, and mental health values in PCOS[31]. However, the cause-effect relationship remains unclear. Our results were similar to those reported in the literature. In other studies, the sole effect of PCOS on QOL could not be evaluated due to the obesity factor. Obesity itself is a concept that affects the QOL.

Physical function and physical role limitation subscales of SF-36 in the present study also showed physical performance[31]. Core endurance and cardiovascular endurance are already indicators of physical performance. These subscales showed physical performance because cardiovascular endurance and core endurance are associated with these subscales. Also, there was a relationship between the psychological subparameter of SF-36 and VO2max in PCOS. This may indicate that mental well-being can positively affect aerobic performance.

The strength of the present study was that it was the first study to present a comprehensive summary of the parameters affected

by PCOS in women of normal weight. Other strengths of the study were its adequate sample size, practical evaluation of the factors, and cost advantage. Also, another strength is the inclusion of only PCOS women with phenotype D for the study group.

Study Limitations

The present study had some limitations. One study limitation was that it did not analyze habitual physical activity levels for work and leisure. Also, nutritional habits and psychological problems could not be evaluated in detail. However, more detailed methods require more time. Also, long-term study protocols can reduce women' adaptability.

Conclusion

PCOS is among the most common endocrine disorders in the world. It is an important health problem that can significantly affect many factors. PCOS treatment and evaluation parameters have gained importance because of the chronic course of PCOS. In PCOS, the evaluation of parameters, such as aerobic capacity, muscular endurance, and QOL, can prevent potential negative problems. Also, determining the factors affected by PCOS may indicate that different approaches can be used as treatment options. According to phenotypes, future studies should investigate the effect of different exercise approaches on these parameters (muscle endurance, VO2max, body awareness, others).

Ethics

Ethics Committee Approval: Permission was granted from the Clinical Research Ethics Committee of the University before the study (decision no: 2017-KAEK-189_2020.01.08_04). All participants were informed about the study based on the 1975 Helsinki Declaration.
Informed Consent: All participants also signed informed consent that they agreed to participate in the study.
Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: H.D., M.M.Ç., Design: H.D., Data Collection or Processing: H.D., M.M.Ç., Analysis or Interpretation: M.M.Ç., Writing: H.D.
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Factors associated with livebirth in couples undergoing their first in vitro fertilization cycle: An internally validated prediction model

İlk IVFsiklusunda canlı doğumla ilişkili faktörler: İnternal doğrulanmış bir prediksiyon modeli

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Abstract

Objective: The aim of the study is to create a new model to predict successful outcome in assisted reproductive techniques.

Materials and Methods: A retrospective cohort study was conducted in tertiary fertility center between 2010 and 2017. Nulliparous women younger than 45 years-old undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) for the first time were included; frozen embryo transfers, canceled induction cycles, freeze-all cycles were excluded. Two prediction models were built using multivariate logistic regression with a subset of the dataset and then were internally validated using bootstrapping methods.

Results: Four hundred eighty eight women were included with 136 (27.9%) live births. The basal model was built using variable age, antral follicle count (AFC), and basal luteinizing hormone (LH) levels. Age over 37 years [odds ratio (OR): 0.07, 95% confidence interval (CI): 0.00-0.36] and AFC below 5 (OR: 0.15, 95% CI: 0.02-0.53) was associated with poorer outcomes whereas an LH level above 6 mIU/mL (OR: 2.24, 95% CI: 1.27-3.94) was associated with better outcomes. Optimism adjusted area under the curve (AUC) of this model was 0.68 (95% CI: 0.62-0.74). Combined model in addition to basal model variables included the length of induction cycle, the endometrial thickness at the day of transfer, grade and count of the transferred embryo. Cycles lasting more than ten days (OR: 2.23, 95% CI: 1.17-4.42), an endometrial thickness greater than 9 mm (OR: 2.07, 95% CI: 1.00-4.53) were associated with better outcomes. Optimism adjusted AUC of this model was 0.76 (95% CI: 0.70-0.81). Calibration of both models was good according to Hosmer Lemeshow test (p=0.979 and p=0.848, respectively).

Conclusion: This internally validated prediction model has good calibration and can be used predicting outcomes in first time IVF/ICSI cycles with modest sensitivity.

Keywords: Prediction models, assisted reproductive techniques, live birth, in-vitro fertilization

Öz

Amaç: Çalışmanın amacı yardımcı üreme tekniklerinde başarılı sonucu tahmin edebilmek için yeni bir model oluşturmak.

Gereç ve Yöntemler: 2010-2017 yılları arasında üçüncü basamak infertilite merkezinde retrospektif bir kohort çalışması yapılmıştır. İlk kez tüp bebek/intrasitoplazmik sperm enjeksiyonu (IVF/ICSI) uygulanan 45 yaş altı nullipar kadınlar dahil edilmiştir; dondurulmuş embriyo transferleri, iptal edilen indüksiyon siklusları, freeze-all sikluslar hariç tutulmuştur. Veri kümesinin bir alt kümesiyle çok değişkenli lojistik regresyon modeli kullanılarak iki tahmin modeli oluşturuldu ve ardından bootstrapping yöntemleri kullanılarak internal olarak doğrulandı.

Bulgular: Yüz otuz altı (%27,9) canlı doğum yapan 488 kadın dahil edildi. Bazal model, yaş, antral folikül sayısı (AFC) ve bazal lüteinize edici hormon (LH) seviyeleri değişkenleri kullanılarak oluşturulmuştur. Otuz yedi yaş üstü [olasılık oranı (OO): 0,07, %95 güven aralığı (GA): 0,00-0,36] ve 5'in altındaki AFC (OO: 0,15, %95 GA: 0,02-0,53) daha kötü sonuçlarla ilişkilendirilirken, LH seviyesinin 6 mIU/mL'nin üzerinde olması (OO: 2,24, %95 GA: 1,27-3,94) daha iyi sonuçlarla ilişkilendirildi. Bu modelin iyimserliğe göre ayarlanmış eğrinin altındaki alanı (AUC) 0,68 (%95 GA: 0,62-0,74) idi. Bazal

PRECIS: Prediction of IVF success can be estimated using baseline characteristics and cycle-specific variable with better precision and calibration compared to traditional models such as templeton.

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model değişkenlerine ek olarak birleşik model, indüksiyon döngüsünün uzunluğunu, transfer gününde endometriyal kalınlığı, transfer edilen embriyonun derecesini ve sayısını içermiştir. On günden fazla süren sikluslar (OO: 2,23, %95 GA: 1,17-4,42), endometriyal kalınlığın 9 mm'den büyük (OO: 2,07, %95 GA: 1,00-4,53) olması daha iyi sonuçlarla ilişkilendirildi. Bu modelin iyimserliğe göre ayarlanmış AUC'si 0,76'dır (%95 GA: 0,70-0,81). Hosmer-Lemeshow testine göre her iki modelin de kalibrasyonu iyiydi (sırasıyla p=0,979 ve p=0,848).
Sonuç: Bu internal olarak doğrulanmış tahmin modeli iyi bir kalibrasyona sahip olmakla birlikte ilk kez IVF/ICSI yapılan hastalarda sonuçları tahmin etmek için iyi bir hassasiyetle kullanılabilir.
Anahtar Kelimeler: Tahmin modeli, yardımcı üreme teknikleri, canlı doğum, in vitro fertilizasyon

Introduction

Subfertile couple counseling is one of the most important parts of assisted reproductive technology (ART) treatment. Several prediction models in the literature provided a data-driven perspective to both clinicians and patients[1-3]. Live birth is the ultimate goal of ART treatment, and the most common and recognized models by Nelson and Lawlor[4] and Templeton et al.[5] used a live birth as the primary outcome. However, both models underestimate the live birth rate based on external validation studies[6]. Another external validation study concluded that a better calibration was achieved for both models after adjustments based on current trends of in vitro fertilization (IVF) success; however, the Templeton model underestimated and the Nelson model overestimated the chances of live birth[7].

Delaying conception attempts and pregnancy until the later ages of a childbearing period is one of the most common causes of increased IVF/intracytoplasmic sperm injection (ICSI) uptake[8]. The success rates of ART treatments for women at advanced age were remarkable. Based on human fertilization and embryology authority reports, even women aged 40-42 years have a higher chance of live birth in 2018 than those aged under 35 in 1991 (11% vs 9%, respectively). The same authority reports concluded that the average age for an IVF cycle was older in 2017 than in 1991 (35.5 vs 33.5, respectively)[9]. These increasing success rates with an older patient population are explained with the individualized cycle management, novel techniques for embryo transfer protocols, and ART laboratory evaluations[9]. With improvements, prediction models are updated based on newer approaches to rationalize the usage of these tools.

This study primarily aimed to establish a well-calibrated model, which combined both patient demographics, cycle management, and embryo transfer day characteristics. The secondary aim is to estimate the live birth rates and compare the Templeton model with the present prediction model.

Materials and Methods

This was a retrospective cohort study conducted in a single tertiary infertility clinic in the Department of Obstetrics and Gynecology at Ankara University. Dataset was gathered from patients evaluated between January 2010 and January 2017. The study was approved by the Institutional Clinical Research Ethics Committee (date: April 25, 2016; number: 08-341-16). Women under 45 years old with fresh embryo cycles were included. All included cycles underwent ICSI. The frozen embryo cycles, women with prior IVF/ICSI cycles, patients with secondary infertility, canceled cycles due to a nonviable sperm during testicular sperm extraction, cycles with >2 transferred embryos, and patients with donor sperm or egg, were excluded. Hospital records from patient files were used to create an anonymous dataset for internal validation. These records were searched manually by E.K. and A.G. Age, infertility duration, hysterosalpingography evaluation notes, body mass index (BMI), infertility indication, and ovarian reserve assessment at day 3 of menstrual cycle were used as patient demographics, and total gonadotropin dose, cycle duration, drugs used for ovarian induction, and sonographic assessments of the follicles and endometrium were used as cycle characteristics. Endometrial thickness, embryo quality, and embryo age were used as transfer characteristics.

After an initial assessment of patients with a detailed historical examination, semen analysis based on the World Health Organization criteria, ovarian reserve, and tubal patency assessment; ovarian stimulation was started during days 3 and 5 of the menstrual cycle. The starting dose was individualized based on patient age, ovarian reserve, and BMI. Further adjustment was also individualized based on the ovarian response assessment. The planned antagonist protocols (Cetrotide, Merck-Serono) were started after 5 days of gonadotropin usage or at least a 12 mm diameter of follicles were seen. Patients with a high risk of ovarian hyperstimulation syndrome were triggered with a dual trigger method or Gonadotrophin-releasing hormone (GnRH) agonists. Vaginal progesterone at 90 mg/day (Crinone 8% gel; Merck-Serono, Istanbul, Turkey) was used for luteal phase support from the day of embryo transfer to 12 weeks of gestational age. The ongoing pregnancy was defined as a pregnancy completed >20 weeks of gestational age. Antenatal follow-ups were organized based on the Ministry of Health guidelines.

Statistical Analysis

Descriptive statistics of all variables used in the study were investigated. The distribution properties of variables were evaluated with the Shapiro-Wilk test and were assumed with a normal distribution feature if the p value was >0.05. Theoretical quantile-quantile graphs of parameters with normal distribution properties (Shapiro-Wilk test p>0.05) were created

and the distribution assumption was visually tested. The variable presentation was made in the form of median value and interquartile range, and specific presentation types were not used for distribution assumption.

T-test or Wilcoxon rank-sum test was used following the distribution assumption of the examined variable for binary group comparisons. Logistic regression analysis was used to create the prediction model. First, all examined parameters were modeled alone and relative probability ratios, confidence interval (CI), and p-values were found. A variable selection model was applied to create the multiparameter model. Step models are prone to produce biased or incompatible clinical reality models, thus all combinations of clinically important parameters or parameters that are important in the univariate regression analysis ($p<0.25$) were tested. The Akaike criterion was used as an aid in parameter selection, and the accuracy and calibration of the model were tested in each step[10,11]. The accuracy of the created models was tested with receiver operating characteristic curves. Model calibration was tested with the Hoslem-Lemeshow test and calibration curves[12]. A certain part of dataset was used to create the model in the study (60%) and all dataset was included in the validation stage (60% + 40%). The internal validation of the model was done using 10,000 different datasets created using the bootstrapping method. The deviation corrected CI of the parameters used for internal validation were found and operating characteristic curves were created with corrected optimism. All statistical analysis R for Windows: Software language for statistical computing (Version 3.1.3) and packages of the same program "pROC," "ModelGood," "rms," "caret," "boot," "ggplot2," and "ROC632" were used. Unless otherwise stated, 0.05 was accepted as the statistically significant p-value limit[13,14].

Results

A total of 488 women who started controlled hyperstimulation for their first embryo transfer were included in the present study. The missing values other than basic patient characteristics were below 1% in the whole dataset. No imputation was applied to the dataset.

Among 488 cycles, 136 (27.9%) resulted in an ongoing pregnancy. The model was based on the comparison of the main characteristics of 305 patients [live birth (number): 85, failed cycles (number): 220], which were presented in Table 1. Age, antral follicle count, day 3 serum luteinizing hormone (LH) level, gonadotropin induction duration, mature oocyte total count, fertilization rate, endometrial thickness on the embryo transfer day, and transferred embryo grade were significantly different between patients with and without live birth.

Selection of Parameters for Univariate Regression Analysis and Prediction Model

Patient demographics, cycle, and transfer day characteristics were put into the one-way regression analysis. All parameters with plausible associations ($p<0.250$) were tested. The cut-off values for age, infertility duration, induction duration, day 3 serum LH level, endometrial thickness, and the total number of retrieved oocytes were visually determined based on the probability distribution graphs. Significant changes were observed in over 37 years of age, over 10 years of infertility duration, over 6 mL/IU for serum LH level, over 9 mm of endometrial thickness, and below four retrieved oocytes. Limit values were used for further regression analysis. Among the parameters examined are age above 37 years ($p=0.032$), low antral follicle number ($p=0.004$), basal LH levels above 6 mIU/mL ($p=0.001$), stimulation cycle lasting longer than 10 days ($p=0.003$), <500 pg/mL estradiol level ($p=0.077$) on the triggering day, <4 collected oocytes ($p=0.013$), and grade B embryo transfer ($p=0.005$) The significance levels were not obtained in other parameters; however; parameters with known clinical effects on live birth were prioritized with the prediction model creation.

Multivariate Regression Analysis and Creation and Calibration of the Predictive Model

Two separate prediction models were created. In the basal model, only the patient demographics were used. In the combined model, cycle and embryo transfer characteristics were included in the patient demographics. One parameter was added or subtracted at a time. The model accuracy and calibration were tested at each stage. Parameters without a significant model consistency increase or that impair its calibration were excluded. The patient age, basal antral follicle count, and day 3 serum LH level were used in the basal model (Table 2). The probability of success decreased (odds ratio: 0.07, 95% CI: 0.00-0.36) in patients aged over 37 years, success rate decreased (odds ratio: 0.15, 95% CI: 0.02-0.53) in <5 antral follicles, and LH greater than 6 mIU/mL (odds ratio: 2.24, 95% CI: 1.27-3.94) was associated with success. The accuracy of the tested basal model revealed an area under the curve (AUC) of 0.68 (95% CI: 0.62-0.74) and model sensitivity of 0.28 (95% CI: 0.17-0.39) for a 10% fixed false-positive rate (Figure 1). The calibration curve of the basal model revealed that the observed probabilities were consistent with the predicted probabilities. The Hosmer-Lemeshow test revealed that the model calibration was good under this observation ($p=0.979$).

In addition to the basal model parameters, the duration of the stimulation cycle, the endometrial thickness, and the number and grade of the embryo transferred were used in the combined model creation. A clinical and statistical interaction was found between the number and grade of embryos, thus, it was adapted to the model considering this feature. Cycles with 10 days or longer duration (odds ratio: 2.23, 95% CI: 1.17-4.42) and endometrial thickness wider than 9 mm (probability ratio: 2.07, 95% CI 1.00-4.53) were more successfully observed. The transferred embryo characteristics revealed a significantly increased successful single grade B embryo transfer, which was found as a negative effect (odds ratio: 0.07, 95% CI 0.00-0.39). The accuracy of the combined model revealed an AUC of 0.76

Table 1. Comparison of basic variables used to establish prediction model

Patient demographics	Live birth (n=85)	No live birth (n=220)	P†
Age (years), median (IQ)	29.0 (26.00-32.0)	31.0 (27.0-36.0)	0.004
Body Mass Index (kg/m^2), median (IQR)	22.4 (21.1-26.2)	23.8 (21.6-26.6)	0.132
Infertility duration (years), median (IQR)	5.0 (3.0-6.0)	5.0 (3.0-8.0)	0.664
Tubal occlusion			
- Unilateral, n (%)	2 (2.4)	7 (3.2)	0.999
- Bilateral, n (%)	4 (4.7)	8 (3.6)	0.743
Infertility etiology			
- Male factor, n (%)	28 (32.9)	93 (42.3)	0.152
- Female factor, n (%)	14 (16.5)	43 (19.5)	0.624
internal validation.	43 (50.6)	84 (38.2)	0.052
Antral follicle count, n (%)			
- ≥5 follicle	62 (72.9)	130 (59.1)	0.025
- <5 follicle	2 (2.4)	38 (17.3)	<0.001
- Polycystic ovaries	21 (24.7)	52 (23.6)	0.881
Day 3 serum FSH level (mIU/mL), median (IQR)	7.0 (6.0-9.0)	7.2 (6.0-9.1)	0.864
Day 3 serum estradiol level (pg/mL), median (IQR)	44.0 (29.5-56.0)	40.5 (30.0-54.0)	0.973
Day 3 serum LH level (mIU/mL), median (IQR)	5.3 (3.7-7.0)	5.0 (3.2-6.0)	0.045
Controlled ovarian hyperstimulation (COH) characteristics			
Initial gonadotropin dosage (IU), median (IQR)	225.0 (225.0-300.0)	225.0 (225.0-300.0)	0.894
Down-regulation method			
- Antagonist, n (%)	82 (96.5)	200 (90.9)	0.144
- Agonist, n (%)	3 (3.5)	20 (9.1)	
Type of gonadotropin for ovarian stimulation			
Only HMG, n (%)	23 (27.1)	73 (33.2)	0.337
- Only recombinant FSH, n (%)	12 (14.1)	25 (11.4)	0.558
- Recombinant FSH and HMG, n (%)	50 (58.8)	122 (55.4)	0.609
Ovarian trigger agent			
Only GnRH agonist, n (%)	7 (8.2)	21 (9.6)	0.827
- Only hCG, n (%)	63 (74.1)	167 (75.9)	0.767
GnRH agonist and hCG, n (%)	15 (17.7)	32 (14.5)	0.485
Total gonadotropin dosage (IU), median (IQR)	2400 (1800-3000)	2400 (2025-2925)	0.566
The number of >17 mm oocytes at trigger day, median (IQR)	3.0 (2.0-4.0)	3.0 (2.0-4.0)	0.242
COH duration (day), median (IQR)	12.0 (11.0-12.0)	11.0 (10.0-12.0)	0.003
Estradiol level at trigger day (pg/mL), median (IQR)	2018 (1370-3196)	1729 (993-2868)	0.071
Progesterone level at trigger day (ng/mL), median (IQR)	0.54 (0.41-1.00)	0.67 (0.47-1.05)	0.331
LH level at trigger day (mIU/mL), median (IQR)	2.0 (1.2-3.5)	2.0 (1.0-3.5)	0.571
OPU and ET characteristics			
Total picked up oocytes, median (IQR)	10.0 (6.0-14.0)	8.0 (5.0-13.0)	0.007
Mature (MII) oocyte number, median (IQR)	9.0 (4.0-12.0)	5.0 (3.0-10.0)	<0.001

Table 1. Continued

	Live birth (n=85)	No live birth (n=220)	P†
Fertilization rate (%), median (IQR)	69.2 (46.4-89.9)	50.0 (33.3-80.0)	0.007
Endometrial thickness at transfer day mm, median (IQR)	11.0 (10.0-12.0)	10.0 (9.0-12.0)	0.001
Embryo transfer number			
- Single, n (%)	65 (76.5)	151 (68.6)	0.206
- Double, n (%)	20 (23.5)	69 (31.4)	
Transferred embryo grade			
- Grade A, n (%)	83 (97.6)	185 (84.1)	<0.001
- Grade B, n (%)	2 (3.4)	35 (15.9)	
Transferred embryo age (day)			
Day 3 embryo, n (%)	66 (77.6)	184 (83.6)	0.246
Day 5 embryo, n (%)	19 (22.4)	36 (16.4)	

IQR: Interquartile range, HMG: Human menopausal gonadotropin, FSH: Follicle-stimulating hormone, GnRH: Gonadotropin-releasing hormone, hCG: Human chorionic gonadotropin, OPU: Oocyte-pick up, ET: Embryo transfer
†Wilcoxon t-test or Fisher's exact test

Table 2. Odds ratios of models created based on multivariate logistic regression analysis

	Odds ratio (OR) (95% confidence interval (CI)	P†
Basal model (AUC: 0.68, 95% CI: 0.62-0.74)		
Age		
- ≤37 age	Reference	
- >37 age	0.07 (0.00-0.36)	0.011
Basal antral follicle count		
- ≥5 follicle	Reference	
- <5 follicle	0.15 (0.02-0.53)	0.012
- Polycystic ovaries	0.60 (0.32-1.09)	0.102
Day 3 serum LH level >6.0 mIU/mL	2.24 (1.27-3.94)	0.004
Combined model (AUC: 0.76, 95% CI: 0.70-0.81)		
Age		
- ≤37 age	Reference	
- >37 age	0.10 (0.00-0.55)	0.030
Basal antral follicle count		
- ≥5 follicle	Reference	
- <5 folikül	0.23 (0.03-0.96)	0.026
- Polycystic ovaries	0.50 (0.25-0.96)	0.043
Day 3 serum LH level >6.0 mIU/mL	2.36 (1.30-4.32)	0.004
COH duration		
- Shorter than 10 days	Reference	
- 10 days or longer	2.23 (1.17-4.42)	0.055
Endometrial thickness at transfer day >9 mm	2.07 (1.00-4.53)	0.016
Transferred embryo grade and number		
- Single, Grade A	Reference	
- Double, Grade A	0.90 (0.45-1.77)	0.697
- Single, Grade B	0.07 (0.00-0.39)	0.014
- Double, Grade B	0.39 (0.02-2.65)	0.456

AUC: Area under curve, CI: Confidence interval
†Multivariate logistic regression

(95% CI: 0.70-0.81) and model sensitivity of 0.31 (95% CI: 0.20-0.42) for a 10% fixed false-positive rate (Figure 2). The consistency of the combined model was statistically significantly higher than the baseline model (AUC: 0.76 vs AUC: 0.68, p<0.001 De Long test, respectively). The calibration curve of the combined model revealed that the observed probabilities were consistent with the predicted probabilities. The Hosmer-Lemeshow test revealed a good model calibration following this observation (p=0.848).

Nomograms were created for the practical application of the models (Supplementary Figure 1,2). The values from the lines next to the parameters are marked first to use the nomogram. Each parameter score is calculated with the lines drawn perpendicular to the score curve above. After the scores are collected, the total score is marked in the total score line below and the possibility of live birth is read with the perpendicular line drawn below.

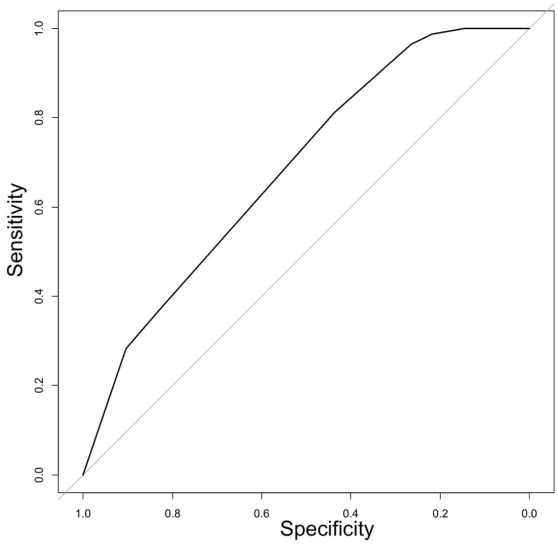


Figure 1. Receiver operating characteristic curve of the basal model using parameters of age, antral follicle count, and luteinizing hormone level

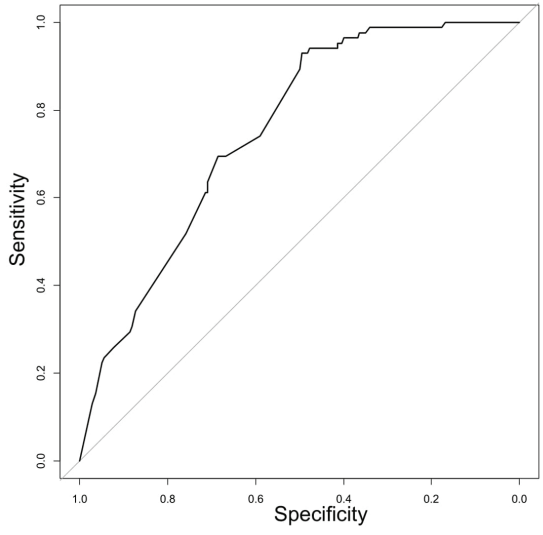
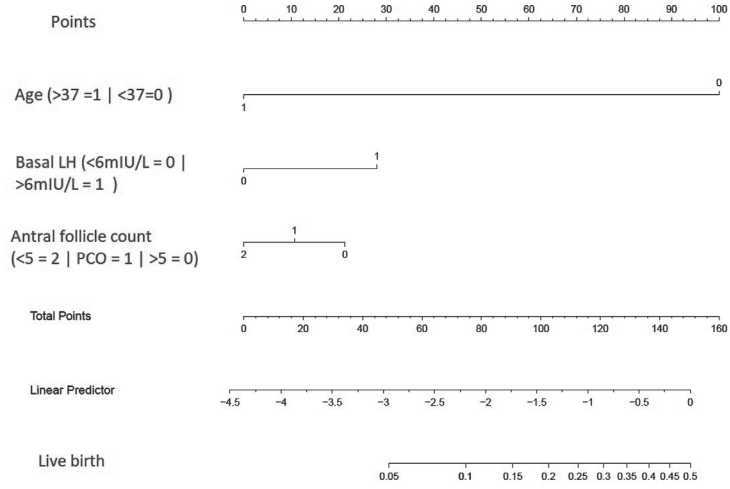


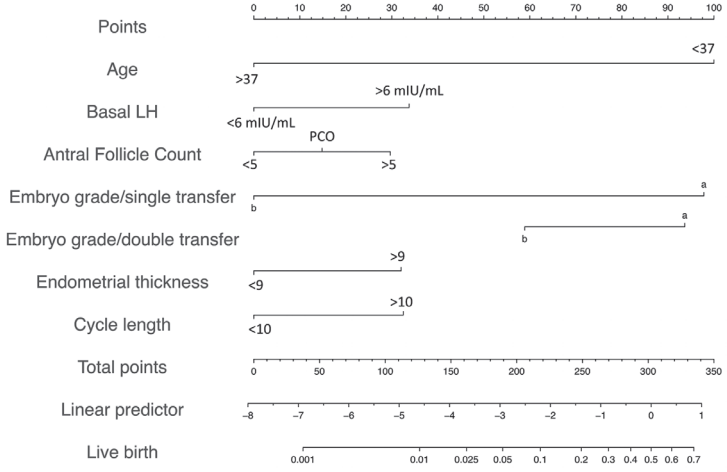
Figure 2. Receiver operating characteristic curve of the basal model using the parameters of age, antral follicle number, luteinizing hormone level, cycle duration, endometrial thickness, and embryo quality and number

The Comparison Between the Templeton and the Present Models

A comparison was made with the Templeton model to show the practical benefit of the basal model. The Templeton model parameters were adapted to our dataset, and receiver operating characteristic curves were created for both the models. The AUC of the Templeton model was 0.60 (95% CI: 0.53-0.67) (Figure



Supplementary Figure 1. The nomogram of the basal model for clinical use. The probability of live births corresponding to the total score observed in the lowest row after the corresponding scores are added on the upper score sheet for each parameter



Supplementary Figure 2. The nomogram of the combined model for clinical use. The probability of live births corresponding to the total score observed in the lowest row after the corresponding scores are added on the upper score sheet for each parameter

3). The accuracy of the Templeton model was lower (p=0.062, DeLong test) than that of the basal model (Figure 3). The sensitivity of the Templeton model for a fixed 10% false positivity rate was very low for clinical use (0.10, 95% CI: 0.03-0.19).

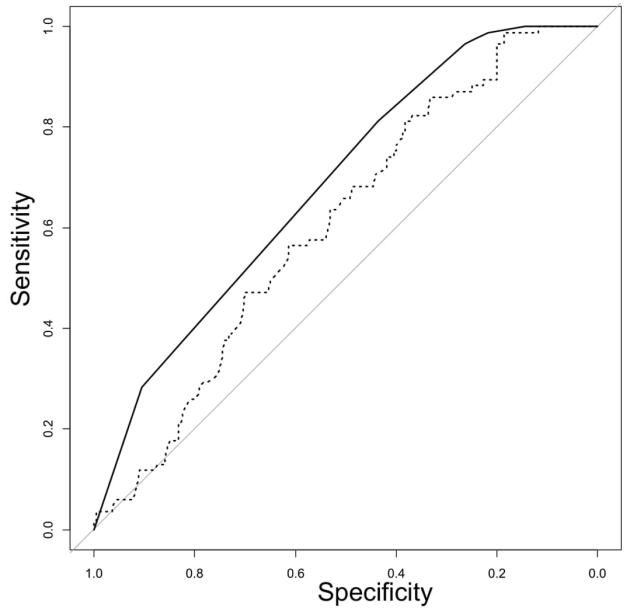


Figure 3. Comparison of the receiver operating characteristic curve of the Templeton model (dashed line) with the curve of our basal model (solid line). The higher predictive accuracy of the present model was demonstrated by the De Long test (p=0.062)

Discussion

The prediction models in the present study have acceptable precision and good calibration. The baseline model was used in the pretreatment phase while informing patients or making treatment decisions. In addition, static (Supplementary Figures 1,2) nomograms are available for practical use of the model, especially for clinicians.

More than 30 prediction models were presented in the literature, wherein most used similar parameters for model creation. Infertility duration and infertility type were not found to be associated with the odds of live birth in this model compared with more recognizable models. One of the reasons is the time censored nature of the infertility duration, which was inevitably affected by the patients' age and the unpredictable exact duration. A recent meta-analysis by van Loendersloot et al.[3] found a weak association between the infertility duration and live birth (odds ratio: 0.99 95% CI: 15 0.98-1.00), which also concluded that among 21 external validation studies, only the model could be generalized, which includes female age, number of retrieved oocytes, developmental stage score, and morphology score of two best embryos. In the present prediction model, in addition to these parameters, day 3 serum LH level, and endometrial thickness were found significantly associated with the odds of live birth.

The most recent prediction model in the literature aimed to calculate "the number of mature oocytes required to obtain at least one euploid embryo"[15]. This model was externally validated and revealed >80% positive predictive values with all the predicted used possibilities by the authors[15,16]. The primary ending for the IVF/ICSI cycle outcome was different than our model; however, female age, sperm source used for ICSI, and the number of mature oocytes were used as parameters of the predictive model. In this study, testicular sperms were used as a sperm source in all of the included cycles to overcome the negative effects of the malefactors.

Endometrial preparation for successful implantation was another key phase of any cycle's endpoint[17,18]. Several factors revealed the functionality of the endometrium. The optimal endometrial thickness was 10 mm at the Vaegter's prediction model, and the impact of endometrial thickness on the models was also similar to ours[19]. Other than the endometrial thickness, the duration of gonadotropin induction showed significance on our model and is a possible indicator of ovarian and endometrial response. Day 3 serum LH level was an important parameter in this study. In two recent studies, basal serum LH level was highly associated with an ovarian response especially agonist protocols[20,21]. Lower LH levels during cycles are also related to a lower ongoing pregnancy rate, and ongoing pregnancy rates are higher at protocols that are supported with a recombinant LH based on a Cochrane review[22].

Stimulation durations longer than 10 days were associated with better outcomes in the first cycles, which is a reflection of the ovarian reserve and its effect on cycle success. Poor responders usually have a short stimulation duration due to already high endogenous follicle-stimulating hormone levels and asynchronous follicle growth. These patients have poorer outcomes compared to normo- and high-responders and our results reflect this mechanism.

Finally, transferred embryo grade and number were associated with ongoing pregnancy rates. This is an expected finding and was established in the literature.

Study Limitations

Several limitations were encountered in this study. Firstly, the number of included patients was below the average from similar studies in the literature. However, considering the patient volume of the clinic where the study was conducted and the included patient group, the number of patients was kept as high as possible and a wide range of years was chosen. In addition, the number of live births (n=85) in the cohort in which the model was developed is above the minimum number (n=10) per parameter in the logistic models[23]. Therefore, the problem is not encountered in terms of statistical power. In addition, a possibility of selection bias is due to its retrospective nature. The possibility of a selection bias is never completely excluded although restrictive exclusion criteria were not set since the data source of the research was the patient files with

complete records. Another limitation was the indicator used for an ovarian reserve. The only parameter was the number of antral follicles and some studies reported that the serum anti-Müllerian hormone (AMH) level reflects the ovarian reserve better. The predictive value of the AMH level was not evaluated since AMH was not a routine parameter recorded in the years in which research records were obtained in our clinic. The internal validation of the developed model was made by developing a mixed-method due to the limited number of patients. The reserved patient population is mixed with the cohort in which the model was developed, and the validation study that performed with the bootstrapping method is more insufficient than the studies using the external cohort. Finally, some interventional procedures were reported with IVF success association, and the relationship of these factors was not studied in our patient population.

The main strength of this study was the patients treated with current IVF protocols and techniques. Given that the prediction models perform best in populations with characteristics similar to the developed cohorts, our model was expected to perform better in external validation studies compared to its historical counterparts. Since the parameters used in the model were easily measured and generally recorded in IVF cycles, no technical problems were expected in external validation studies. In addition, during the creation and testing of the model, the highest standard statistical practices were adhered to, and the model was created with careful attention to technical principles. The value of the area (0.76) remaining in the high curve quotation observed in our study is the result of careful parameter selection and good statistical practice. Finding static and dynamic nomograms for the practical use of our model was another strong aspect.

Conclusion

The present created model was well-calibrated and easily interpretable to routine IVF/ICSI cycles. The combined model aid in the informed decision phase of the fertility-seeking couples; however, external validation is necessary with a large-sized prospective cohort to confirm the clinical usage.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Clinical Research Ethics Committee (date: April 25, 2016; number: 08-341-16).
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.E.Ş., B.Ö., M.S., C.B., R.A., Concept: E.K., B.B., Design: E.K., B.B., Data Collection or Processing: E.K., C.B., A.G., Analysis or Interpretation: E.K., B.B., Literature Search: E.K., C.B., A.G., Y.E.Ş., Writing: E.K., C.B., B.B.

Conflict of Interest: The authors report no conflict of interest.
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Review / Derleme

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Are women with polycystic ovary syndrome more vulnerable to COVID-19 infection?

Polikistik over sendromu olan kadınlar COVID-19 enfeksiyonuna daha mı hassaslar?

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Abstract

Severe acute respiratory syndrome-coronavirus-2, the causative virus of Coronavirus disease-2019 (COVID-19), penetrates into the hosts' tissues via binding of its spike protein to the angiotensin converting enzyme-2 (ACE-2) receptors after activation of the hosts' protease enzymes. The most prominent effect is observed when the virus binds to the ACE-2 receptors of the alveolar epithelium and endothelium. Testosterone exhibits an immunosuppressive effect, and androgens play a modulatory role on protease enzymes. It is known that various comorbidities, including obesity; pregnancy; diabetes mellitus (type 1 or type 2); hypertension; cancer; chronic kidney, liver, and lung diseases; cerebrovascular disease; heart conditions; human immunodeficiency virus infection; immunologic disease; and immune suppression; affect the severity of COVID-19 infection. Polycystic ovary syndrome (PCOS) affects 5-10% of reproductive aged-women. Hirsutism is observed in 70-80% of the patients, while increased testosterone levels are detected in more than 50% of the women with PCOS. This syndrome is also associated with hyperandrogenism, insulin resistance, increased renin-angiotensin system activity, diabetes, and metabolic syndrome in a remarkable number of cases. PCOS also manifests a chronic pro-inflammatory state. Hyperandrogenism through hyperinsulinemia causes adipocyte hypertrophy and dysfunction that result in increased secretion of pro-inflammatory adipokine, which culminates in the creation of a chronic inflammatory state. In light of the metabolic and hormonal changes observed in women with PCOS, which make them more susceptible to severe COVID-19 infection, health care givers should provide special care and detailed counseling services.

Keywords: COVID-19, polycystic ovary syndrome, hyperandrogenism

Öz

Şiddetli akut solunum sendromu-koronavirüs-2, spike proteinin konakçının proteaz enzimleri ile aktivasyonu sonrası anjiyotensin dönüştürücü enzim-2 (ACE-2) reseptörlerine bağlanır ve hücre içerisine girer. En önemli etkiler virüsün alveol epiteli ve endoteldeki ACE-2 reseptörlerine bağlanmasından sonra ortaya çıkar. Testosteronun immün sistemi baskılayıcı etkisi vardır ve androjenler proteaz enzimleri üzerinde düzenleyici rol oynar. Obezite, gebelik, diabet (tip 1 veya tip 2), hipertansiyon, kanser, kronik böbrek, karaciğer ve akciğer hastalıkları, serebrovasküler hastalıklar, kalp hastalıkları, insan bağışıklık yetmezliği virüsü enfeksiyonu, immünolojik hastalıklar, immünosüpresyon başta olmak üzere eşlik eden diğer hastalıklar Koronavirüs hastalığı-2019 (COVID-19) enfeksiyonunun ciddiyetini artırmaktadır. Polikistik over sendromu (PKOS) üreme çağındaki kadınların %5-10'unu etkiler, hastaların %70-80'inde hirsutizm, %50'den fazlasında ise artmış testosteron düzeyleri saptanır. Bu sendrom hastaların önemli bir kısmında ayrıca hiperandrojenizm, insülin rezistansı, artmış renin-anjiyotensin sistemi aktivitesi, diyabet, metabolik sendrom ile ilişkilidir. PKOS ayrıca kronik pro-enflamatuvar bir durum gösterir. Hiperandrojenim hiperinsülinemi yoluyla adipositlerde hipertrofi ve fonksiyon bozukluğuna neden olarak pro-enflamatuvar adipokin sekresyonu ve kronik enflamatuvar bir duruma yol açar. PKOS'li kadınlarda onları ciddi COVID-19 enfeksiyonuna daha duyarlı hale getiren hormonal ve metabolik değişikliklerin ışığında sağlık hizmet sunucuları özel bir bakım ve ayrıntılı bir danışmanlık hizmeti sunmalıdır.

Anahtar Kelimeler: COVID-19, polikistik over sendromu, hiperandrojenizm

Introduction

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), infects multiple organs, especially the alveolar epithelium, thereby causing severe acute respiratory distress. Various factors are involved in the pathophysiology and course of the infection, which mainly include high initial viral load, lung damage resulting from the infiltration of increased inflammatory monocyte macrophages (IMMs), neutrophils, and pro-inflammatory cytokines. Thus, severe acute respiratory distress

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develops, which is accompanied by the activation of endothelial cells that cause pulmonary thrombosis[1]. Replication of the virus results in massive inflammatory mediator release, and an increased inflammatory response is related to the severity of the disease.

SARS-CoV-2 is a single-stranded RNA virus. The spike (S) protein, which is among the four structural proteins, including S, envelope, membrane, and nucleocapsid proteins, has an affinity for the angiotensin converting enzyme-2 (ACE-2) receptors on human cells. This enables the binding and penetration of the virus into the human cells after priming of the S proteins by host proteases, such as transmembrane serine protease 2 (TMPRSS2), furin, and cathepsin L. Increased affinity and increased expression of the ACE-2 receptor allows greater transmission of the virus into the host[2,3]. The binding of CoV-2 to the ACE-2 receptor leads to the downregulation of this receptor and detoriates its protective effect against cardiovascular disease and acute respiratory distress[4]. The most prominent effect is observed when the virus binds to the ACE-2 receptors of the alveolar epithelium and endothelium.

The COVID-19 pandemic has affected more than 150 million people worldwide, causing more than three million deaths; however, a separate data for women and men has not been reported for most countries. Gender has been proposed as one of the risk factors in COVID-19 infection because there is a remarkable difference between men and women in terms of mortality and morbidity[5]. A national Danish study revealed a 50% increased risk of mortality and severe morbidity related to COVID-19 infection in men as compared with women, regardless of age and presence of comorbidities[6]. Studies from China, South Korea, and the United States reported similar or sometimes higher prevalence in women, depending on the criteria applied for COVID-19 testing, whether it is a community testing or symptomatic peoples' testing[7,8]. However, it has been reported that the incidence of severe disease and death was higher among men.

Various studies have shown that comorbidities and health conditions that affect the severity of COVID-19 infection include obesity; pregnancy; diabetes (type 1 or type 2); hypertension; cancer; chronic kidney, liver, and lung diseases; cerebrovascular disease; sickle cell disease or thalassemia; dementia or other neurological conditions; Down syndrome; heart conditions; human immunodeficiency viral infection; immunological disease; immune suppression; smoking; and substance use[8-10]. The differences between male and female ACE-2 receptor expression is questioned in order to understand different clinical outcomes in women and men during COVID-19 infection[11] besides other factors such as differences in immunological response and the effect of sex steroids on the immunological response[12,13]. Testosterone suppresses immune response, and androgens have a modulatory effect on proteins that facilitate the entry of SAR-CoV-2 into hosts' tissues.

Wambier and Goren.[14] mentioned that the hyperandrogenic phenotype in men, which manifests itself in form of androgenic alopesia, acne, and oily skin, increasingly makes the chest and face hair more vulnerable to COVID-19 infection. In an animal study, male and female mice were infected with SARS-CoV-2 and it was found that the male mice had higher mortality and increased accumulation of IMMs and neutrophils in the lungs. Moreover, gonadectomy or antiandrogens did not improve mortality in male mice. However, increased IMMs were encountered in ovariectomized or antiestrogen-treated female mice[15]. Increased IMMs cause elevated lung cytokine/chemokine levels, vascular leakage, and impaired T-cell response[15].

Polycystic ovary syndrome (PCOS) affects 5-10% of reproductive aged-women, and hirsutism is observed in 70-80% of the patients[16], while increased testosterone levels have been detected in more than 50% of the women with PCOS[17]. Hirsutism in PCOS is associated with both elevated levels of androgen, which is mainly secreted from the ovary, and increased sensitivity of the pilosebaceous unit to androgens[18]. Hyperandrogenism through hyperinsulinemia causes adipocyte hypertrophy and dysfunction that result in increased secretion of proinflammatory adipokine and creation of a chronic inflammatory state. PCOS is also associated with insulin resistance, central obesity, metabolic syndrome, and diabetes mellitus[19]. Obesity accompanies PCOS in a remarkable proportion of the patients. A study comparing the association of obesity with the severity of COVID-19 infection in men and women revealed that class II and III obesity (35-39.9 kg/m^2 and ≥40 kg/m^2, respectively) were independent risk factors of in-hospital deaths in men and in women that was observed only in class III obesity. In-hospital deaths were also found to be associated with IL-6 levels in obese patients[8]. This might be related to the different fat distribution between men and women, considering that men had an androgenic distribution of fat, which is mainly a central adiposity, the type encountered in women with PCOS. Adipocyctes secrete pro-inflammatory cytokines that facilitate chronic inflammatory response.

The risk of venous thromboembolism increased up to 1.5-fold in women with PCOS[20]. Androgens modulate proteases, mainly the TMPRSS2, furin, and cathepsin L, which play a major role in the binding and penetration of the virus into hosts' tissue[21]. Huffman et al.[18] investigated the effects of androgens on SARS-CoV-2 viral entry proteins in hyperandrogenic female mice treated with dihydrotestosterone (DHT) after detecting androgen receptors in the lung, kidney, brain, left ventricle, gastrointestinal system, and tibialis anterior of the untreated female mice. This study demonstrated the upregulatory effect of androgens in hyperandrogenic female mice on COVID-19 priming proteins and the authors suggested that this mechanism might explain the aggravated cardiac, renal, and gastrointestinal symptoms in COVID-19-infected women with PCOS. Subramanian et al.[22] conducted a population-

based study in England and reported that the crude COVID-19 incidence among 21,292 women with PCOS was 18.1, whereas this rate was 11.9 per 1.000 persons/year among 78,310 women without PCOS after age and body mass adjustment. Adjusting women with PCOS were found to have an increased risk of 28%. Morgante et al.[23] stated that besides the presence of insulin resistance linked to hyperandrogenism, another risk factor in hyperandrogenic women with PCOS was higher activity of androgen receptors and renin-angiotensin system. Hyperglycemia, obesity, and chronic inflammatory state were other risk factors besides the high incidence of vitamin D deficiency in women with PCOS[24]. Vitamin D plays an important role in immunoregulatory mechanisms due to its pivotal role in decreasing cytokine storm by decreasing the secretion of pro-inflammatory cytokines[25].

The overlaping risk factors for PCOS and COVID-19 infection should be considered because women with PCOS are at a higher risk for contracting severe COVID-19 infection. Therefore special care and detailed counseling should be provided for women with PCOS.

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Review / Derleme

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A meta-analysis for the risk and prevalence of preeclampsia among pregnant women with COVID-19

COVID-19'lu gebe kadınlarda preeklampsi riski ve prevalansı: Bir meta-analiz

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Abstract

Preeclampsia and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection are both life-threatening disorders when they occur during pregnancy. They are similarly characterized by systemic immune activation and have a deleterious effect on maternal endothelial cells. During the coronavirus disease-2019 (COVID-19) pandemic, there were reports of preeclampsia or a preeclampsia-like syndrome occurring in pregnant women with SARS-CoV-2 infection. We performed a meta-analysis to estimate the risk and prevalence of preeclampsia and SARS-CoV-2 infection in pregnant women. A comprehensive literature search was conducted in PubMed, Web of Science, Scopus, and China National Knowledge Infrastructure to identify all relevant studies published up to February 29, 2020. All studies that reported the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection were selected. A total of 10 case-control studies and 15 case series met our inclusion criteria. Pooled data revealed no significant difference between infected pregnant women and uninfected pregnant women for the risk of preeclampsia [odds ratio (OR)=1.676, 95% confidence interval (CI) 0.679-4.139, p=0.236]. The stratified analysis revealed significant risk in the infected Asian pregnant women (OR=2.637, 95% CI 1.030-6.747, p=0.043), but not Caucasian. The prevalence of preeclampsia was 8.2% (95% CI 0.057-0.117) in infected pregnant women with COVID-19 in the overall population. Its prevalence was highest in North America (10.7%), followed by Asian (7.9%), Caucasian (6.7%), European (4.9%), and West Asian (2.6%) infected pregnant women. Our pooled data showed that the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection was 8.2%. However, there was no increased risk of occurrence of preeclampsia among pregnant women with SARS-CoV-2 infection.
Keywords: SARS-CoV-2, COVID-19, preeclampsia, pregnancy, hypertensive disease of pregnancy, meta-analysis

Öz

Preeklampsi ve şiddetli akut solunum yolu sendromu koronavirüsü-2 (SARS-CoV-2) enfeksiyonu, gebelikte ortaya çıktıklarında hayatı tehdit eden bozukluklardır. İkisi de benzer şekilde sistemik immün aktivasyon ile karakterizedir ve maternal endotel hücreleri üzerinde zararlı bir etkiye sahiptir. Koronavirüs hastalığı-2019 (COVID-19) pandemisi sırasında, SARS-CoV-2 enfeksiyonu olan hamile kadınlarda preeklampsi veya preeklampsi benzeri bir sendrom oluştuğuna dair raporlar mevcuttur. Burada, hamile kadınlarda preeklampsi ve SARS-CoV-2 enfeksiyonu riskini ve prevalansını tahmin etmek için bir meta-analiz gerçekleştirdik. 30 Şubat 2020'ye kadar yayınlanan tüm ilgili çalışmaları belirlemek için PubMed, Web of Sciences, Scopus ve Çin Ulusal Bilgi Altyapısı'nda kapsamlı bir literatür taraması yapıldı. SARS-CoV-2 enfeksiyonlu hamile kadınlarda preeklampsi prevalansıyla ilgili tüm çalışmalar seçildi. Toplam 10 olgu kontrol çalışması ve 15 olgu serisi dahil etme kriterlerimizi karşıladı. Toplanan veriler, enfekte hamile kadınlar ile enfekte olmayan hamile kadınlar arasında preeklampsi riski açısından anlamlı bir fark olmadığını ortaya koydu [risk oranı (RO=1,676, %95 güven aralığı (GA) 0,679-4,139, p=0,236]. Tabakalı analiz, enfekte Asyalı hamile kadınlarda (RO=2,637, %95 GA 1,030-6,747, p=0,043) anlamlı risk ortaya çıkardı, ancak Kafkasyalılarda

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bulunmadı. Genel popülasyonda COVID-19 ile enfekte hamile kadınlarda preeklampsi prevalansı %8,2 (%95 GA 0,057-0,117) idi. Prevalans en yüksek Kuzey Amerika'da (%10,7) olup, bunu Asya (%7,9), Kafkasya (%6,7), Avrupa (%4,9) ve Batı Asya (%2,6) izlemekteydi. Birleştirilmiş verilerimiz, SARS-CoV-2 enfeksiyonlu gebe kadınlarda preeklampsi prevalansının %8,2 olduğunu gösterdi. Bununla birlikte, SARS-CoV-2 enfeksiyonu olan hamile kadınlar arasında preeklampsi oluşma riskinde artış görülmemiştir.
Anahtar Kelimeler: SARS-CoV-2, COVID-19, preeklampsi, gebelik, gebeliğin hipertansif hastalığı, meta-analiz

Introduction

Hypertensive disorders of pregnancy are common complications that put mothers and their fetuses at heightened risk for perinatal morbidity and mortality in addition to life-long sequelae and long-term risk of cardiovascular disease[1]. Preeclampsia is the most frequent hypertensive complication of pregnancy, occurring in approximately five to seven percent of pregnancies globally, with a higher incidence in some indigenous women and those from low- and middle-income countries, such as those in sub-Saharan Africa[2,3]. In addition to its being an obstetrical management challenge, preeclampsia is also a major global maternal health and public health problem as it is responsible every year for over 70,000 maternal deaths and 500,000 fetal deaths worldwide. In the United States, preeclampsia is a leading cause of maternal death, severe maternal morbidity, maternal intensive care admissions, cesarean sections, low birth weight and fetal growth restriction, preterm rupture of membranes, and prematurity[2,4]. It also accounts for up to 18% of maternal deaths in the United States annually[5]. As a multisystem disease, preeclampsia has many known risk factors, including obesity, primiparity, renal disease, chronic hypertension, advanced maternal age, multiple- or molar pregnancy, and pre-gestational- or gestational diabetes mellitus. However, these factors alone do not account for the disease onset. Preeclampsia is considered to have its origin in pathological factors related to placental development, implantation, and defective remodeling of the spiral arteries. These result in uteroplacental and maternal vascular malperfusion accompanied by altered immunoregulation and inflammatory response[6-8].

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been the most critical public health crisis to occur in this century. The effects of this global pandemic will persist for many years[9-11]. As is often the case with an emerging viral disease, pregnant women and their infants have been of great concern, especially since pregnant women with SARS-CoV-2 infection and their infants are at higher risk for poor outcomes than are those who are not pregnant[12].

Several clinical studies reported that COVID-19 is associated with an increased risk of preeclampsia and a preeclampsia-like syndrome in infected pregnant women[13-15], but their results remain controversial[16,17]. COVID-19 is also associated with immune activation that results in elevated levels of pro-inflammatory cytokines, including interleukin (IL)-2, IL-6, IL-7, and tumor necrosis factor-α[18]. Although it is considered a respiratory disease primarily, SARS-CoV-2 infection also affects endothelial cells, and during pregnancy, it can lead to endotheliitis, microthrombi deposition, and microvascular dysfunction[19]. As of January 22, 2020, there were over 47,096 confirmed COVID-19 cases during pregnancy, with 58 related deaths in the United States[20]. Some studies have suggested that preeclampsia may be more common in pregnant women with COVID-19 than other adverse outcomes[19]. Thus, we performed a meta-analysis to estimate the risk and prevalence of preeclampsia and SARS-COV-2 infection in pregnant women.

Materials and Methods

Publication Search

Ethical approval or patient consent was not needed because this is a meta-analysis, and all data were extracted from published literature. We performed a comprehensive literature search in PubMed, Web of Knowledge, Web of Science, Embase, Scientific Information Database, WanFang, VIP, Chinese Biomedical Database, Scientific Electronic Library Online, and the China National Knowledge Infrastructure database to collect all relevant studies published up to February 29, 2020. Combinations of the following keywords were used in the search: ("COVID-19 virus disease" OR "severe acute respiratory syndrome coronavirus 2" OR "SARS-CoV-2" OR "2019 novel coronavirus infection" OR "2019-nCoV infection" OR "coronavirus disease" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "COVID-19 virus infection") AND ("preeclampsia" OR "Preeclampsia toxemia" OR "toxemia" OR "hypertrophic decidual vasculopathy" OR "gestational hypertension" OR "pregnancy-associated hypertension") AND ("Risk" OR "Prevalence" OR "Incidence" OR "Frequency" OR "Prevalence"). Moreover, the reference list of the retrieved studies and reviews were manually checked to identify more potentially eligible studies. The search was conducted in English, Chinese, and Persian. When overlapping data on the same cases were included in more than one publication, only the one with the larger sample size was selected.

Selection Criteria

The inclusion criteria for these studies included: 1) case-control and case series studies; 2) studies reporting the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection; 3) studies published in English and Chinese; 4) detailed data for estimating the odds ratio (OR) and 95% confidence interval (CI), and available allele genotype frequencies for cases and controls. The exclusion criteria were as follows: 1) studies not describing the incidence of preeclampsia in pregnant women with SARS-CoV-2 infection; 2) Studies not providing usable or sufficient data for pooling; 3) studies focusing on animals or ***in***

vitro; 4) abstracts, comments, conference abstracts, editorials, reviews, meta-analysis; and 7) duplicated studies or data.

Data Extraction

Two authors extracted the data from included studies and verified the accuracy of the data, and a third author resolved discrepancies. The following data were extracted from each article: first author name, year of publication, ethnicity (Asian, Caucasian, African, and mixed populations), country of origin, diagnostic methods, number of pregnant women with SARS-CoV-2 infection and healthy women, and number of preeclampsia in infected women. If selected articles did not report the necessary data, the corresponding authors were contacted by email to request the missing data.

Statistical Analysis

All statistical calculations were performed using Comprehensive Meta-Analysis software version 2.0 (Biostat, USA). Two-sided p-values <0.05 were considered statistically significant. The ORs and 95% CIs were used to assess the risk and prevalence of preeclampsia in pregnant women with COVID-19. The significance of the pooled OR was determined by the Z-test, and a value of $p<0.05$ was regarded as statistically significant. The between-study heterogeneity was identified with the Q-test and I^2 index (range, 0% to 100%), where $p\leq0.10$ indicated significant heterogeneity. I^2 values of >50% indicated heterogeneity among studies. The random-effects model (DerSimonian-Laird method) was applied to calculate the pooled OR and 95% CI if there was obvious heterogeneity among the studies. Otherwise, we used a fixed-effect model (Mantel-Haenszel method) for the meta-analysis. Stratified analyses were performed according to ethnicity, genotyping methods, and sources of controls. A sensitivity analysis was performed to assess the effects of individual studies on pooled results and the stability of the results. We used Egger's and Begg's tests to evaluate publication bias, with $p>0.05$ as evidence for no potential publication bias. The trim and fill method was also applied to detect publication bias. All tests were two-sided, and $p<0.05$ was considered statistically significant.

Results

Characteristics of Selected Studies

As shown in Figure 1, our initial search yielded 620 studies, with duplicate studies removed, resulting in 252 studies remaining. Among them, 121 studies were excluded based on titles and abstracts. The selection criteria excluded 106 studies. Finally, 25 publications, including 10 case-control studies[21-30] and 15 case series[31-45], were selected. Their basic information and preeclampsia distributions for case-control studies and case series are presents in Tables 1 and 2. These studies included 2039 pregnant women with SARS-CoV-2 infection (with 121 preeclampsia) and 15,834 healthy women (with 1126 preeclampsia) in case-control studies, and 2021 pregnant women with SARS-CoV-2 infection with 98 preeclampsia) in the case series. The publication year of all selected studies was 2020. The majority of study patients came from the Mainland China (n=10), followed by United States (n=4), Spain (n=2), Sweden (n=1), France (n=1), Canada (n=1), Turkey (n=1), Iran (n=1), Peru (n=1), Kuwait (n=1), and India (n=1). The individual study sample sizes ranged from 5 to 1285 (Table 1). Of the 23 studies, 21 studies used real-time polymerase chain reaction (qRT-PCR) to diagnose SARS-CoV-2 infection, one study used a combination of PCR and chest CT. One study used only a serum antibody test.

Quantitative Data Synthesis

Risk

The summaries of risk for preeclampsia in pregnant women with SARS-CoV-2 infection are shown in Table 3. The pooled data revealed that SARS-CoV-2-infected pregnant women had no significant risk in the occurrence of preeclampsia (OR=1.676, 95% CI 0.679-4.139, p=0.236, Figure 2A) compared with non-infected pregnant women. However, the stratified analysis showed a significant risk among infected Asian pregnant women (OR=2.637, 95% CI 1.030-6.747, p=0.043, Figure 2B), but not among Caucasian (OR=1.335, 95% CI 0.436-4.089, p=0.613, Figure 2B), Chinese (OR=2.437, 95% CI 0.628-9.459, p=0.198), North America (OR=1.296, 95% CI 0.279-6.030, p=0.741), and European (OR=1.771, 95% CI 0.908-3.454, p=0.094) pregnant women with SARS-CoV-2 infection.

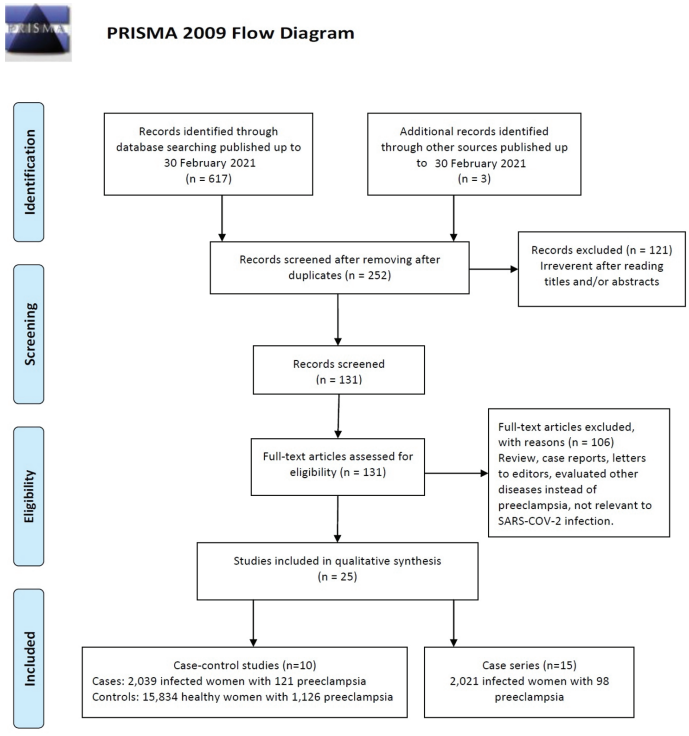

Figure 1. The study selection and inclusion process

Table 1. Details of included studies in the current meta-analysis

First author	City (country)	Ethnicity	Study design	Diagnostic method	Clinical presentation at admission	Confirmed cases	Sever or critical	Preeclampsia	Age group or mean
Adhikari	Texas (US)	Caucasian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	245	13	26	27.0±6.6
Wang	Boston (US)	Caucasian	Case/ Control	PCR	Symptomatic and asymptotic	53	8	10	29.8±5.9
Patberg	New York (US)	Caucasian	Case/ Control	PCR	Symptomatic and asymptotic	77	NA	5	29.9±6.2
Brandt	New Brunswick (Canada)	Caucasian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	61	7	6	30.3±6.4
Ahlberg	Karolinska (Sweden)	Caucasian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	155	NA	12	32.1±4.9
Egerup	Copenhagen (Denmark)	Caucasian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	1285	NA	53	28.6-34.7
Pirjani	Babol (Iran)	West Asian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	66	NA	6	30.97±6.38
Yang	Hubei (China)	East Asian	Case/ Control	qRT-PCR and CT	Symptomatic and asymptotic	65	NA	1	NA
Li	Hubei (China)	East Asian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	16	0	1	26-37
Zhang	Hubei (China)	East Asian	Case/ Control	qRT-PCR	Symptomatic and asymptotic	16	1	1	NA
London	New York (US)	Caucasian	Case Series	qRT-PCR	Symptomatic and asymptotic	55	0	3	24-38
Sahin	Ankara (Turkey)	Caucasian	Case Series	qRT-PCR	Symptomatic and asymptotic	533	7	5	28.04±5.84
Sentilhes	Strasbourg (France)	Caucasian	Case Series	qRT-PCR	Symptomatic and asymptotic	54	17	3	30.6±6.2
Mendoza	Barcelona (Spain)	Caucasian	Case Series	qRT-PCR	Symptomatic	42	6	6	26-37
Perez	Spain	Caucasian	Case Series	qRT-PCR	Symptomatic and asymptotic	82	4	4	19-48
Arroyo	Trujillo (Peru)	Mixed	Case Series	Serologically	Symptomatic and asymptotic	20	4	12	21-45
Chen	Wuhan (China)	East Asian	Case Series	qRT-PCR	Symptomatic	9	0	2	26-40
Chen	Hubei (China)	East Asian	Case Series	qRT-PCR	Symptomatic	5	0	1	25-31
Hu	Wuhan (China)	East Asian	Case Series	qRT-PCR	Symptomatic	6	0	2	26-36
Yang	Wuhan (China)	East Asian	Case Series	qRT-PCR	Symptomatic	7	0	1	NA
Yan	Wuhan (China)	East Asian	Case Series	qRT-PCR	Symptomatic	116	NA	4	24-41
Zhang	Wuhan (China)	East Asian	Case Series	qRT-PCR	Symptomatic	18	1	1	24-34
Cao	Hube i(China)	East Asian	Case Series	qRT-PCR	Symptomatic and asymptotic	10	0	3	30-31
Ayed	Al-Jahra (Kuwait)	West Asian	Case Series	qRT-PCR	Symptomatic	185	22	1	27-34
Mahajan	Mumbai (India)	South Asia	Case Series	qRT-PCR	Symptomatic	879	2	50	24-36

RT-PCR: Real-time polymerase chain reaction, NA: Not available

Prevalence

The summaries of the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection are shown in Table 3. The prevalence of preeclampsia was 8.2% (95% CI 0.057–0.117, Figure 3A) in infected pregnant women overall. The stratified analysis by ethnicity and region showed that the prevalence of preeclampsia infected pregnant women was the highest in North America (10.7%; 95% CI 0.082-0.139), followed by Asian (7.9%; 95% CI 0.046-0.132, Figure 3B), Caucasian (6.7%; 95% CI 0.043-0.104, Figure 3C), European (4.9%; CI 0.026-0.088), and West Asian (2.6%; CI 0.002-0.315) women. Moreover, the stratified analysis by country of origin revealed that the prevalence of preeclampsia among US-American- and Chinese-infected women were 10.8% (CI 0.081-0.143) and 10.4% (CI 0.050-0.201), respectively.

Table 2. Details of selected case-control studies

First Author	Pregnant women with COVID-19		Uninfected pregnant women	
	Total	Preeclampsia	Total	Preeclampsia
Adhikari	245	26	3035	939
Wang	53	10	760	59
Patberg	77	5	56	0
Brandt	61	6	122	10
Ahlberg	155	12	604	26
Egerup	1285	53	28	1
Pirjani	66	6	133	4
Yang	65	1	10930	83
Li	16	1	121	0
Zhang	16	1	45	4

COVID-19: Coronavirus disease-2019

Heterogeneity Test

In this meta-analysis, there was a significant difference between-study heterogeneity for risk (I^2=84.05; P_H≤0.001) and prevalence (I^2=81.54; P_H≤0.001) in the overall population. Therefore, we performed stratified analyses by ethnicity to explain the potential source of heterogeneity. Results showed that the heterogeneity disappeared in the subgroup analysis among Asian, Chinese, and European women for preeclampsia risk and among North American women for prevalence, indicating that ethnicity might be the major source of heterogeneity in this study (Table 3).

Table 3. Summary for the risk and prevalence of preeclampsia in pregnant women with COVID-19

Subgroup	Type of model	Heterogeneity		Odds Ratio				Publication bias	
		I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
Risk									
Overall	Random	84.04	≤0.001	1.676	0.679-4.139	1.120	0.236	0.858	0.049
Caucasian	Random	89.11	≤0.001	1.335	0.436-4.089	0.506	0.613	1.000	0.181
Asian	Fixed	8.059	0.353	2.637	1.030-6.747	2.022	0.043	1.000	0.719
North American	Random	91.64	≤0.001	1.296	0.279-6.030	0.331	0.741	0.734	0.276
European	Fixed	0.00	0.664	1.771	0.908-3.454	1.677	0.094	NA	NA
Chinese	Fixed	19.34	0.289	2.437	0.628-9.459	1.287	0.198	1.000	0.364
US	Random	93.99	≤0.001	1.405	0.180-10.950	0.325	0.745	1.000	0.504
Prevalence									
Overall	Random	81.54	≤0.001	0.082	0.057-0.117	-12.101	≤0.001	0.779	0.263
Caucasian	Random	82.01	≤0.001	0.067	0.043-0.104	-10.828	≤0.001	0.350	0.615
Asian	Random	58.99	0.004	0.079	0.046-0.132	-8.427	≤0.001	0.541	0.416
North American	Fixed	38.68	0.163	0.107	0.082-0.139	-14.113	≤0.001	0.089	0.122
European	Random	80.17	0.010	0.049	0.026-0.088	-9.146	≤0.001	0.734	0.234
West Asian	Random	85.98	0.008	0.026	0.002-0.315	-2.496	0.013	NA	NA
Chinese	Random	51.37	0.030	0.104	0.050-0.201	-5.434	≤0.001	0.788	0.630
US	Fixed	53.62	0.091	0.108	0.081-0.143	-13.140	≤0.001	0.308	0.627

NA: Not applicable, OR: Odds ratio, CI: Confidence interval

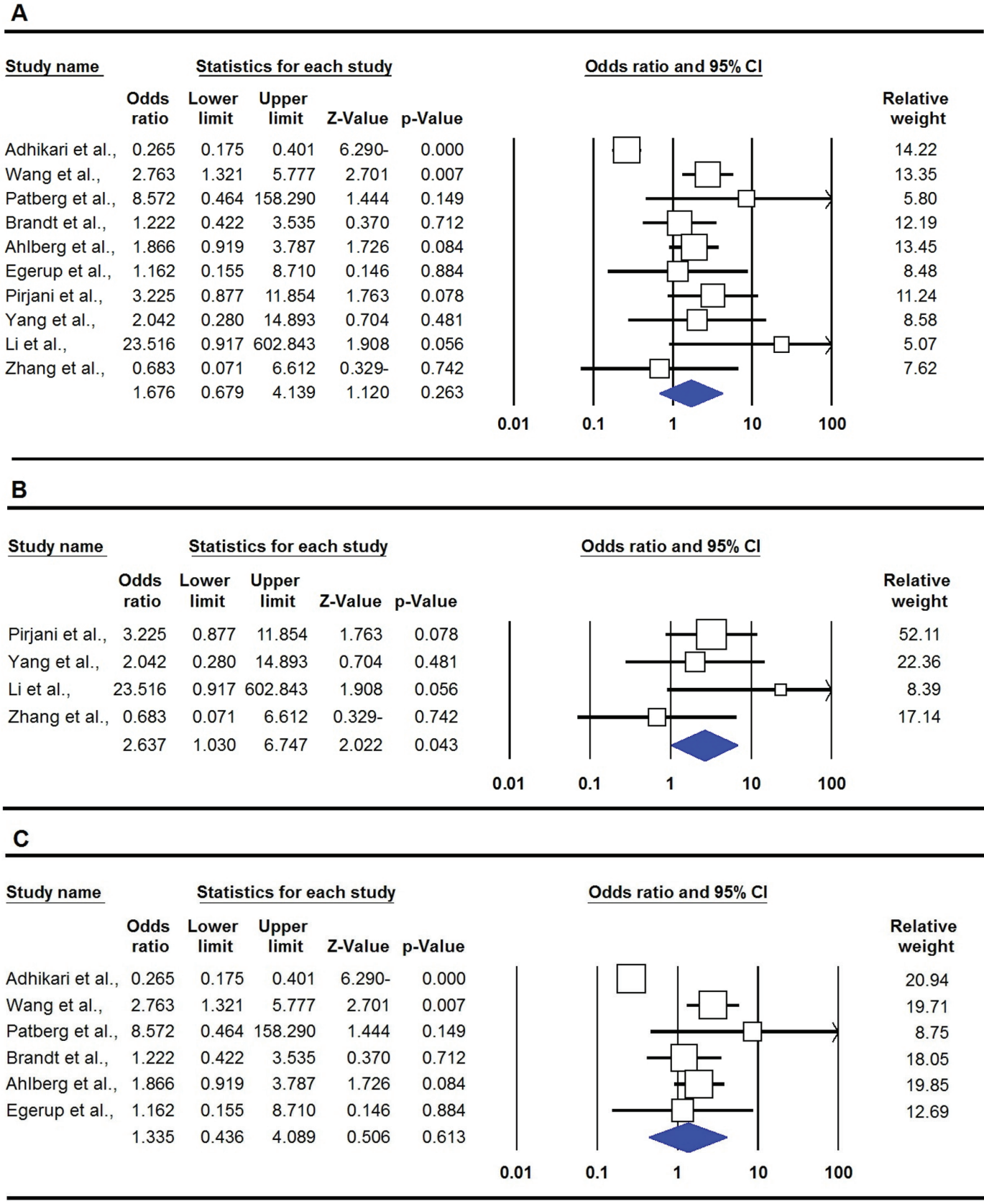

Figure 2. Forest plots for the risk of preeclampsia in pregnant women with SARS-CoV-2 infection. A: overall population; B: Caucasians; and C: Asians

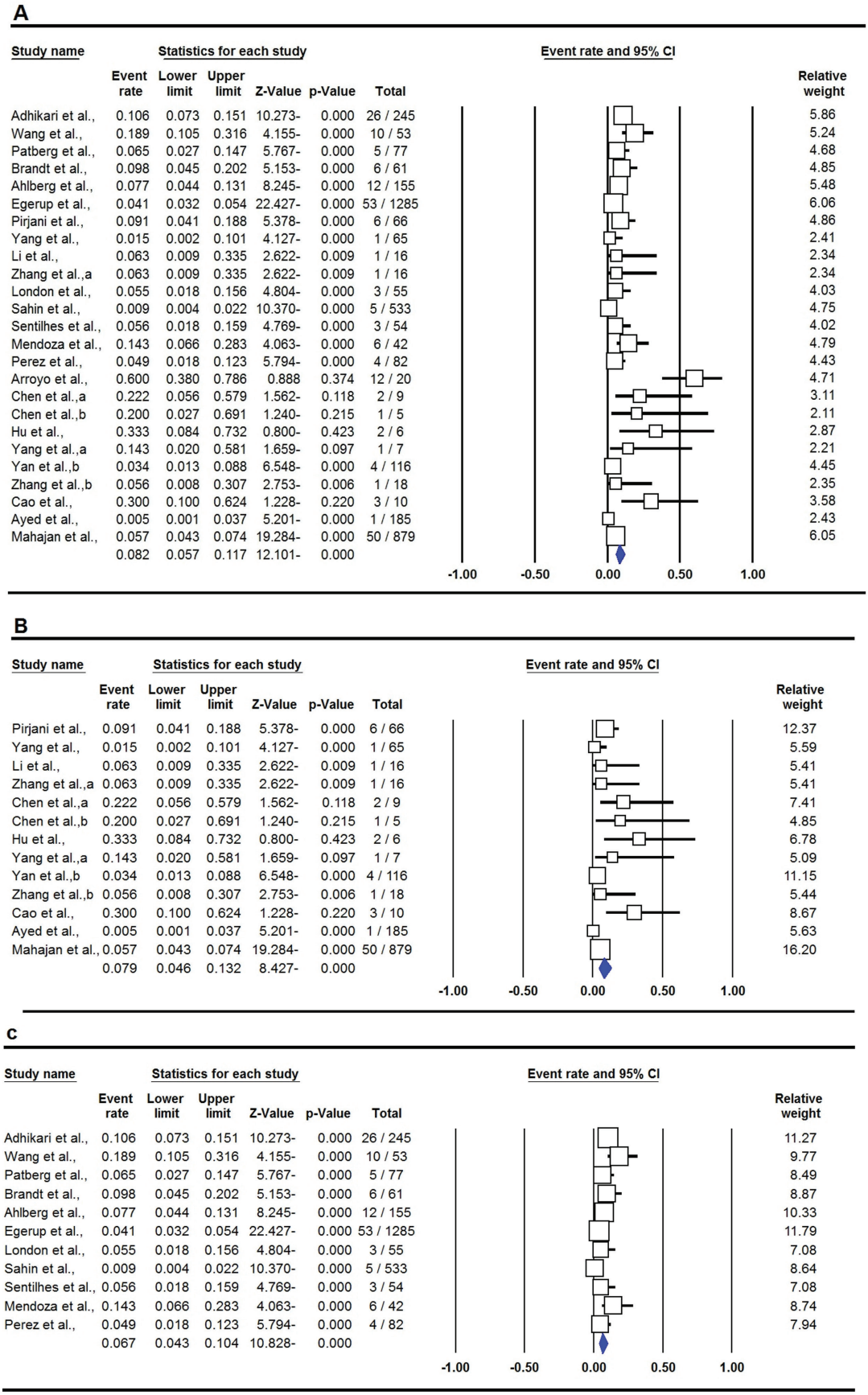

Figure 3. Forest plots for the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection. A: overall population; B: Caucasians; and C: Asians

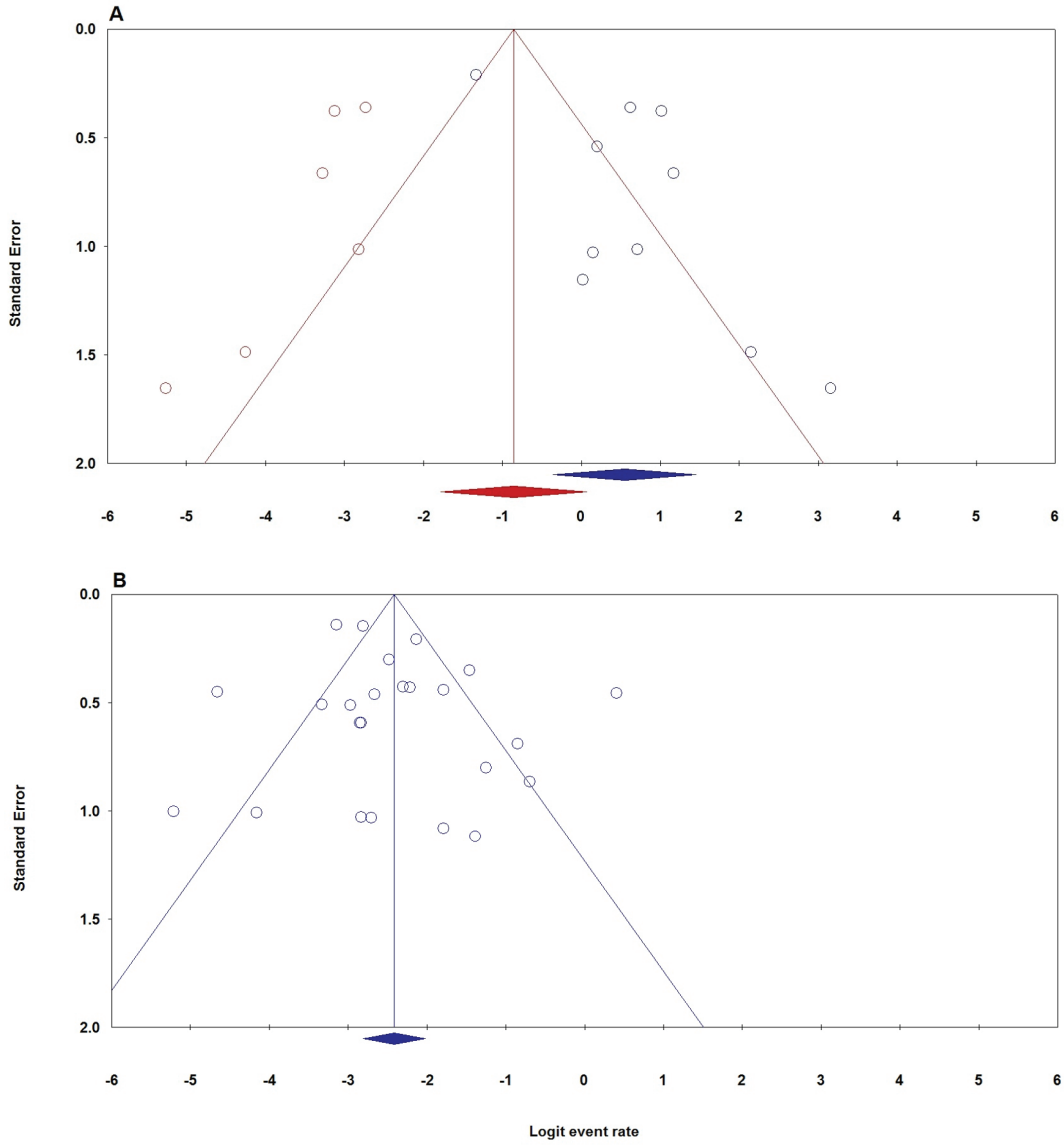

Figure 4. Begg's funnel plot for publication bias test for the risk and prevalence of preeclampsia in pregnant women with SARS-CoV-2. A: Risk; B: Prevalence

Sensitivity Analysis and Publication Bias

We conducted a leave-one-out sensitivity analysis to identify the impact of individual research on the pooled data. The significance of the pooled ORs was not influenced by excluding those studies, indicating that our pooled data were statistically robust. This sensitivity analysis showed that our findings were not dependent on a single study. We also checked for publication bias, and a funnel plot showed symmetrical distribution. Egger's regression test p-value for risk was (P_{Beggs}=0.858; P_{Eggers}=0.049, Figure 4A) and the prevalence (P_{Beggs}=0.779; P_{Eggers}=0.263, Figure 4B) of preeclampsia in pregnant women with COVID-19 (Table 3). Moreover, Begg's funnel plot showed evidence of publication bias for risk of preeclampsia in pregnant women with SARS-CoV-2 infection. Thus, we used the Duval and Tweedie "trim and fill" method to adjust for possible publication bias in the literature for preeclampsia risk. Figure 3 shows the Duval and Tweedie non-parametric "trim and fill" method funnel plot. The results did not change for preeclampsia risk in women with SARS-CoV-2 infection, indicating that our pooled ORs are reliable.

Discussion

The pathogenesis of SARS-CoV-2 infection occurring during pregnancy and its relationship to co-morbid conditions is not well understood[46]. Recently, some studies reported SARS-CoV-2 infection was not associated with a heightened risk of preeclampsia in infected pregnant women[13,31]. Moreover, some of them explained that preeclampsia-like features could be present in some pregnancies with a severe course of COVID-19[13]. Joudi et al.[47] provided the first description of treatment for preeclampsia in a woman with severe manifestations and concurrent COVID-19 disease.

To the best of our knowledge, this is the first meta-analysis examining the risk of preeclampsia in pregnant women with SARS-CoV-2 infection. Our pooled data showed no significant difference in the occurrence of preeclampsia between SARS-CoV-2-infected and uninfected pregnant women. Angiotensin-converting enzyme 2 (ACE2) is implicated in pregnancy complications, such as miscarriage, ectopic pregnancy, and preeclampsia[48]. Bloise et al.[49] reported that the expression of ACE2 or transmembrane protease serine 2 (TMPRSS2) at the decidual interface (placenta and decidua) did not change in pregnancies complicated by preeclampsia. Thus, their results did not show that pregnancies complicated by preeclampsia are at increased risk of placental SARS-CoV-2 infection and vertical transmission. Based on the consecutive case series, the rates of gestational diabetes, hypertensive disorders of pregnancy, and preeclampsia were not higher in pregnant women with COVID-19 than in non-infected pregnant women[50]. In a retrospective analysis of 2682 pregnant women who delivered at a single hospital in Sweden between March 25 and July 24, 2020, Ahlberg et al.[25] reported that 156 women (5.8%) were positive for SARS-CoV-2. They found that pregnant women with SARS-CoV-2 infection had a higher prevalence of preeclampsia than uninfected pregnant women (7.7% vs 4.3%; OR=1.84; 95% CI 1.004-3.36). Moreover, their data demonstrated that SARS-CoV-2 test positivity in women in active labor was associated with a higher prevalence of preeclampsia and a lower prevalence of labor induction. They suggested that COVID-19 is a complex respiratory infection with systemic effects that may resemble preeclampsia.

Our study revealed that the pooled prevalence of preeclampsia was 8.2% (95% CI 0.057-0.117) among pregnant women with SARS-CoV-2 infection. Its prevalence based on ethnicity and region was highest in North American (10.7%), followed by Asian (7.9%), Caucasian (6.7%), European (4.9%), and West Asian (2.6%) infected pregnant women. Moreover, stratified analysis by country of origin revealed that the prevalence of preeclampsia among US-American and infected Chinese women was 10.8% and 10.4%, respectively. The two other medically significant coronavirus pathogens are severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)[51,52]. Both SARS-CoV and MERS-CoV are associated with increased complications, such as preeclampsia[15,53]. Di Mascio et al.[14], in a meta-analysis based on 19 studies, including 79 hospitalized women (41 pregnancies affected by COVID-19, 12 by MERS, and 26 by SARS), described the outcomes of the combined coronavirus spectrum (SARS, MERS, and SARS-CoV-2) in pregnant women. Their results showed that the prevalence of preeclampsia in pregnant women was 16.2% (2/19; 95% CI 4.2-34.1). However, the prevalence could not be reliably attributed to the virus infections alone. It is plausible that such manifestations result from widespread inflammation and endothelial damage, in a process called the "cytokine storm," responsible for many symptoms of coronavirus-related organ injury. Chi et al.[54] performed a meta-analysis on outcomes of pregnant women with COVID-19 showing that four (12.90%) of confirmed COVID-19 patients had preeclampsia. Diriba et al.[55] revealed that preeclampsia was observed among 5.7% of pregnant women infected with COVID-19 and MERS-CoV. Bellos et al.[56] performed a meta-analysis examining maternal and perinatal outcomes in pregnant women with COVID-19, finding that 5.4% (8/148) of infected women had preeclampsia. Mahajan et al.[45], in a study of 879 infected pregnant women with COVID-19 (859 singleton pregnancies and 20 multiple gestation pregnancies), described a higher risk of preeclampsia among women with multiple gestation pregnancies and COVID-19[43].

Mendoza et al.[34] conducted an observational study describing a preeclampsia-like syndrome in 14.3% (6/42) or 6 of 8 (62.5%) pregnant women with severe COVID-19 (at >20 weeks of gestation) who were admitted to the intensive care unit. However, there were no symptoms of preeclampsia among the 34 pregnant women with non-severe forms of COVID-19. Five of these subjects did not have evidence of pre-eclampsia before the diagnosis of severe COVID-19 pneumonia. They explained that pregnant women with severe COVID-19 could develop a preeclampsia-like syndrome, which might be distinguished from actual preeclampsia by sFlt-1/PlGF, LDH, and UtAPI assessment[31]. However, their report should be interpreted with caution because of the observational nature of the study, the small number of pregnant women with severe infection, and the possible role of confounding factors[16]. ACE2 receptors in the placenta might be associated with an increased risk of mother to neonate transmission of the virus[57-59]. It is speculated that the placenta possesses ACE2 receptors on villous cytotrophoblasts and syncytiotrophoblasts. Their high expression at the maternal-fetal interface is dysregulated by SARS-CoV-2 and Poly (U) Specific (ENDOU, placental protein 11 or PP11) might be involved in the high rates of preeclampsia associated with severe COVID-19[60,61]. Bloise et al.[49] reported that pregnancies complicated by preeclampsia are not associated with changes in the expression of ACE2 and TMPRSS2.

Although the current meta-analysis was designed systematically to include all the eligible studies, some limitations need to be mentioned. Most of the included studies were conducted in Caucasian and East Asian pregnant women with SARS-CoV-2 infection, and so the findings may not apply to other racial/ethnic groups. Further studies with larger sample sizes across different racial/ethnic groups are necessary. Second, since our meta-analysis was confined to certain variables, we could not perform any sub-analyses by age, the severity of COVID-19 and preeclampsia, and history of preeclampsia because of the lack of data in primary studies. In addition, due to the small numbers of cases and reporting, we could not specifically address the relationship between SARS-CoV-2 infection and

other hypertensive complications of pregnancy, including eclampsia and HELLP syndrome.

Conclusion

In summary, this study revealed that the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection was 8.2%. Its occurrence among SARS-CoV-2-infected pregnant Asian women was higher than among women of other ethnicities. However, infected pregnant European women had a lower prevalence than seen in other ethnic groups. In contrast, SARS-CoV-2 infection was not significantly associated with an increased risk of preeclampsia in infected pregnant women compared with non-infected pregnant women. Identifying the prevalence of preeclampsia in pregnant women with SARS-CoV-2 infection is essential to provide proper obstetrical management and deliver the necessary critical interventions for pregnant women during the COVID-19 pandemic.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K.Z., S.A.D., A.J., R.S.T., F.F., F.A., Concept: M.K.Z., S.A.D., D.A.S., R.B., Design: D.A.S., R.B., Data Collection or Processing: M.K.Z., D.A.S., R.B., S.A.D., A.J., R.S.T., F.F., F.A., H.N., Analysis or Interpretation: M.K.Z., D.A.S., R.B., S.A.D., A.J., R.S.T., F.F., F.A., H.N., Literature Search: M.K.Z., D.A.S., R.B., S.A.D., A.J., R.S.T., F.F., F.A., H.N., Writing: M.K.Z., S.A.D., R.S.T., H.N.

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Meta-analysis of the frequency of intrauterine growth restriction and preterm premature rupture of the membranes in pregnant women with COVID-19

COVID-19'lu gebe kadınlarda intrauterin büyüme geriliği ve preterm erken membran rüptürünün sıklığı: Bir meta-analiz

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Abstract

The impact of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in pregnancy has yet to be determined. Some studies indicate that SARS-CoV-2 infection may be associated with a higher risk of adverse outcomes in pregnant women. Here, we performed a meta-analysis to estimate the frequency of intrauterine growth restriction (IUGR) and preterm premature rupture of the membranes (PPROM) in pregnant women with Coronavirus disease-2019 (COVID-19). A comprehensive search was performed in various databases, such as PubMed, Scopus, SciELO, MedRxiv, and Web of Science, to find all relevant studies published before 10 February 2021. Cross-sectional and consecutive case series reporting the pregnancy outcomes of COVID-19 were included. A total of 24 studies, including 8 studies on IUGR and 16 studies on PPROM, were selected. Pooled data showed that the frequencies of IUGR and PPROM in pregnant women with COVID-19 were 2.6% and 9.9%, respectively. Analyses stratified by ethnicity showed that the frequencies of IUGR in Asian and Caucasian COVID-19-infected pregnant women were 2.9% and 2.0%, respectively. Moreover, the frequencies of PPROM in Asian and Caucasian COVID-19-infected pregnant women were 10.2% and 5.8%, respectively. This meta-analysis showed that the frequencies of IUGR and PPROM in COVID-19-infected pregnant women were 2.6% and 9.9%, respectively. However, well-designed, large-scale and multicenter clinical studies are required to improve and validate these results.

Keywords: COVID-19, SARS-CoV-2, pregnancy, intrauterine growth restriction, preterm premature rupture of membranes

Öz

Şiddetli akut solunum sendromu-koronavirüs-2'nin (SARS-CoV-2) gebelikteki etkisi henüz bilinmemektedir. Bazı çalışmalar, SARS-CoV-2 enfeksiyonunun hamile kadınlarda artmış olumsuz sonlanım riski ile ilişkili olabileceğini göstermiştir. Burada, Koronavirüs hastalığı-2019'lu (COVID-19) hamile kadınlarda intrauterin büyüme geriliği (İUGG) ve preterm erken membran rüptürü (PERM) sıklığını tahmin etmek için bir meta-analiz gerçekleştirdik. 10 Şubat 2021'den önce yayınlanan tüm ilgili çalışmaları bulmak için PubMed, Scopus, SciELO, MedRxiv ve Web of Science'da kapsamlı bir arama yapıldı. Gebe kadınlarda COVID-19'un gebelik sonuçlarını bildiren kesitsel çalışmalar ve ardışık olgu serileri dahil edildi. İUGG üzerine 8 çalışma ve PERM üzerine 16 çalışma olmak üzere toplam 24 çalışma seçildi. Havuzlanmış veriler, COVID-19'lu enfekte kadınlarda İUGG ve PERM sıklığının sırasıyla %2,6 ve %9,9 olduğunu göstermiştir. Etnik kökene göre tabakalı analizler, Asyalı ve Kafkasyalı enfekte hamile kadınlarda İUGG sıklığının sırasıyla %2,9 ve %2,0 olduğunu göstermiştir. Ayrıca, Asyalı ve Kafkasyalı enfekte gebelerde PERM sıklığı sırasıyla %10,2 ve %5,8 idi. Bu meta-analiz, COVID-19 ile enfekte

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hamile kadınlarda İUGG ve PERM sıklığının sırasıyla %2,6 ve %9,9 olduğunu göstermiştir. Bununla birlikte, bu sonuçları iyileştirmek ve doğrulamak için iyi tasarlanmış, büyük ölçekli ve çok merkezli klinik çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: COVID-19, SARS-CoV-2, gebelik, intrauterin büyüme geriliği, preterm erken membran rüptürü

Introduction

Since December 2019, the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) outbreak has resulted in 120 million cases and over 2.2 million deaths worldwide[1,2]. Several viral infections are known to increase the risk of poor neonatal and maternal outcomes during pregnancy[3-5]. Maternal physiological changes in pregnancy have a significant impact on the coagulation and immune, respiratory, and cardiovascular systems of the fetus and may have positive or negative effects on Coronavirus disease-2019 (COVID-19) disease progression[6]. Some authors have indicated that two previous epidemics of SARS and Middle Eastern respiratory syndrome (MERS-CoV) were associated with adverse pregnancy and neonatal outcomes[7]. Our experiences in pregnancies complicated by infection with the previous coronaviruses have led us to believe that pregnant woman may be vulnerable to the adverse effects of COVID-19. Initial studies on pregnant women have revealed that COVID-19 significantly increases the risk of abortion, preterm birth, stillbirth, intrauterine growth restriction (IUGR), intrauterine death, low birth weight, and case fatality[8,9]. Some studies have also demonstrated that maternal COVID-19 could affect the oxygen supply of the fetus, leading to placental insufficiency, IUGR, fetal distress, and/or fetal demise[10]. Recent epidemiological and clinical studies have reported different results on the maternal and fetus outcomes of COVID-19[11]. The current systematic review and meta-analysis aimed to evaluate these results and determine the frequency of IUGR and PROM in pregnant women with COVID-19.

No definitive evidence of the vertical transmission of SARS-CoV-2 infection from mother to child is available in the existing data, but some pregnancy complications, such as premature birth, IUGR, and spontaneous abortion, through COVID-19-positive mothers have been reported.

Materials and Methods

Search Strategies

We performed a meta-analysis in accordance with the preferred reporting items for systematic reviews and meta-analyses guidelines (http://www.prismastatement.org). This meta-analysis does not contain any studies with human participants performed by any of the authors. Electronic databases, including PubMed/MEDLINE, Europe PMC, Google Scholar, EMBASE, Cochrane Library, SciELO, Springer Link, Technology Journal, Egyptian Knowledge Bank, Chinese Biomedical Database, the China National Knowledge Infrastructure platform, VIP, Chinese literature (Wan Fang), and China science, were comprehensively searched to identify all relevant studies published up to 10 February 2021. Combinations of the following search terms were used (designed using English Medical Subject Headings keywords and Emtree terms): ("COVID-19 virus disease" OR "SARS -CoV-2" OR "SARS-CoV-2" OR "2019 novel coronavirus infection"' OR "2019-nCoV infection" OR "coronavirus disease" OR "coronavirus disease-19" OR "2019-nCoV disease" OR "COVID-19 virus infection") AND ("IUGR" OR "Intrauterine growth retardation" OR "IUGR") AND ("Premature rupture of membranes" OR "Prelabor rupture of membranes" OR "PPROM"). We restricted our search to human studies and articles published in English, Farsi, and Chinese. An extra search was conducted in the reference lists of the included studies to avoid missing eligible studies that had not been identified in the primary search. The Centers for Disease Control and Prevention and World Health Organization websites were also evaluated.

Inclusion and Exclusion Criteria

The primary studies were selected according to the following inclusion criteria: 1) case series, case-control, or cohort studies; 2) studies reporting pregnancy outcomes in pregnant women with SARS-CoV-2 infection; 3) studies with sufficient data to calculate odds ratios (ORs) with 95% confidence intervals (CIs). The following exclusion criteria were also implemented: 1) insufficient data; 2) non-human or in vitro studies; 3) abstracts, case reports, non-consecutive case series, posters, editorials, reviews, conference papers, previous meta-analyses, and non-standard data presentations; and 4) overlapping and duplicated data.

Data Extraction

Two authors independently performed title-abstract screening on all primary studies. The full text of the selected articles was also screened, and all necessary data were extracted into a standardized form. In case of disagreement, a third author was involved to reach a consensus for all items. The following features were extracted for pooled estimation: name of the first author, year of publication, country of origin, ethnicity, total number of pregnant women with COVID-19, and numbers of reported PPROM and IUGR. The corresponding author was contacted by email for any missing data. The studies included in this current meta-analysis did not obviously overlap with the subjects in other studies. If a duplicate publication was found or the same population was used in multiple studies, the publication with the larger sample size was included in the meta-analysis.

Statistical Analysis

The frequency of IUGR and PPROM in pregnant women with SARS-CoV-2 infection was assessed by ORs with 95% CIs. The

significance of pooled ORs was determined using the Z-test; here, p<0.05 defined as the significance threshold. Between-study heterogeneity was tested using the Q-statistic test; p≤0.10 indicated significant heterogeneity crossing studies. The I2 statistic was used to qualify heterogeneity (range, 0-100%: I2=0-25%, no heterogeneity; I2=25-50%, moderate heterogeneity; I2=50-75%, large heterogeneity; I2=75-100%, extreme heterogeneity). If significant heterogeneity (p<0.1) was detected, a random-effects model (i.e., the DerSimonian and Laird method) was selected to pool the data; otherwise, the fixed-effects model (i.e., the Mantel-Haenszel method) was employed. Visual inspection of the funnel plot was used to assess potential publication bias. Moreover, Egger's test was performed to assess the publication bias statistically, and p<0.05 was considered statistically significant. If the publication bias tests indicated bias, the Duval and Tweedie "trim-and-fill" method was used to adjust this bias[12]. All of the statistical calculations were performed using comprehensive meta-analysis version 2.0 software (Biostat, USA). Two-sided p-values <0.05 were considered statistically significant.

Results

As shown in Figure 1, 484 articles were found in different databases and manual searches; these articles were published up to 10 January 2020. Duplicate articles were removed, and 241 articles remained. All of these articles were screened by reading their abstracts/titles, and another 128 studies were eliminated. Of the remaining studies, 89 articles were excluded because they were reviews, meta-analyses, or non-consecutive case reports or did not report the necessary data. Finally, a total of 24 studies, including 8 studies on IUGR with 2.504 infected pregnant women and 60 IUGR cases[13-20] and 16 studies on PPROM with 1.469 infected pregnant women and 91 PPROM cases[15,20-34], were selected. The characteristics of the studies included in the present meta-analysis are presented in Table 1. The publication year of the selected studies was 2020. All selected studies were published in English and Chinese. Among the studies on IUGR, five were performed among Caucasians, two were conducted among Asians and one was conducted among a mixed population. Among the studies on PPROM, nine studies were performed among Asians, four were conducted among Caucasians, and one was conducted among Latin-Americans. The studies were performed in the United States, France, Turkey, Iran, India, Spain, Peru, and China.

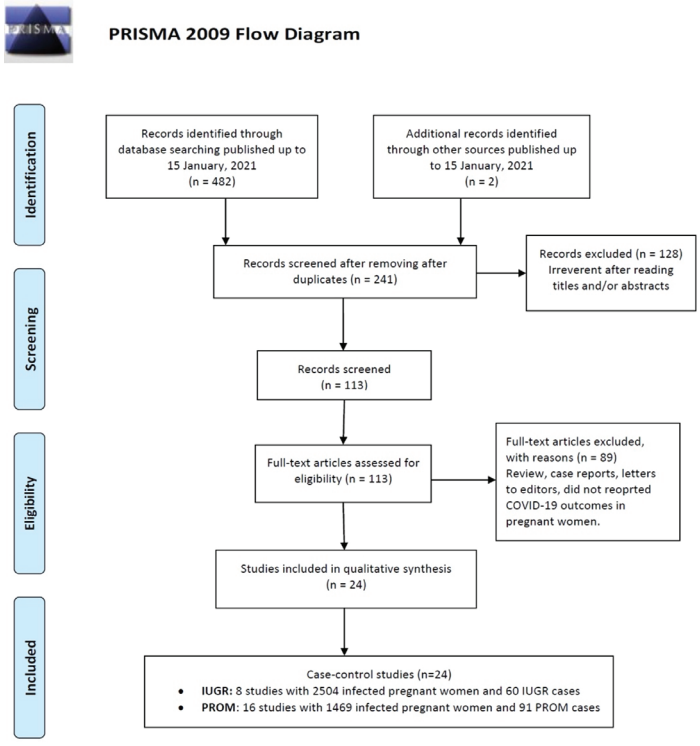

Figure 1. Study selection and inclusion process

IUGR: Intrauterine growth restriction, PPROM: Preterm premature ruptures of membranes, COVID-19: Coronavirus disease-2019

Quantitative Data Synthesis

IUGR

Summaries of the frequency of IUGR in pregnant women with SARS-CoV-2 infection are shown in Table 2. The pooled data showed that the frequency of IUGR in COVID-19-infected women was 2.6% (95% CI: 0.021-0.034, p≤0.001, Figure 2A). Analyses stratified by ethnicity showed that the frequencies of IUGR among Asian and Caucasian pregnant women were 2.9% (95% CI: 0.020-0.042, p≤0.001) and 2.0% (95% CI: 0.014-0.031, p≤0.001, Figure 2B), respectively. Moreover, the frequency of IUGR among North-American women was 2.5% (95% CI: 0.016-0.040, p≤0.001).

PPROM

Table 2 presents the summaries of the frequency of PPROM among SARS-CoV-2-infected pregnant women. The pooled data showed that the frequency of PPROM among pregnant women infected with COVID-19 was 9.9% (95% CI: 0.058-0.164, p≤0.001, Figure 3A). Analyses stratified by ethnicity showed that the frequencies of PPROM among Asian and Caucasian infected pregnant women were 10.2% (95% CI: 0.056-0.181, p≤0.001, Figure 3B) and 5.8% (95% CI: 0.011-0.248, p≤0.001, Figure 3C), respectively. Moreover, the frequency of PPROM among Chinese women was 10.6% (95% CI: 0.072-0.155, p≤0.001).

Between-study Heterogeneity and Sensitivity Analysis

As shown in Table 1, significant between-study heterogeneity was noted in the overall population for PPROM (I2=79.27, PH≤0.001) but not for IUGR (I2=34.02, PH=0.157). Subgroup analysis by ethnicity demonstrated no decrease in heterogeneity. However, subgroup analysis among Chinese women revealed that the country of origin may be a source of heterogeneity in the current meta-analysis (Table 2). We performed a sensitivity analysis to evaluate the stability of the results by sequentially

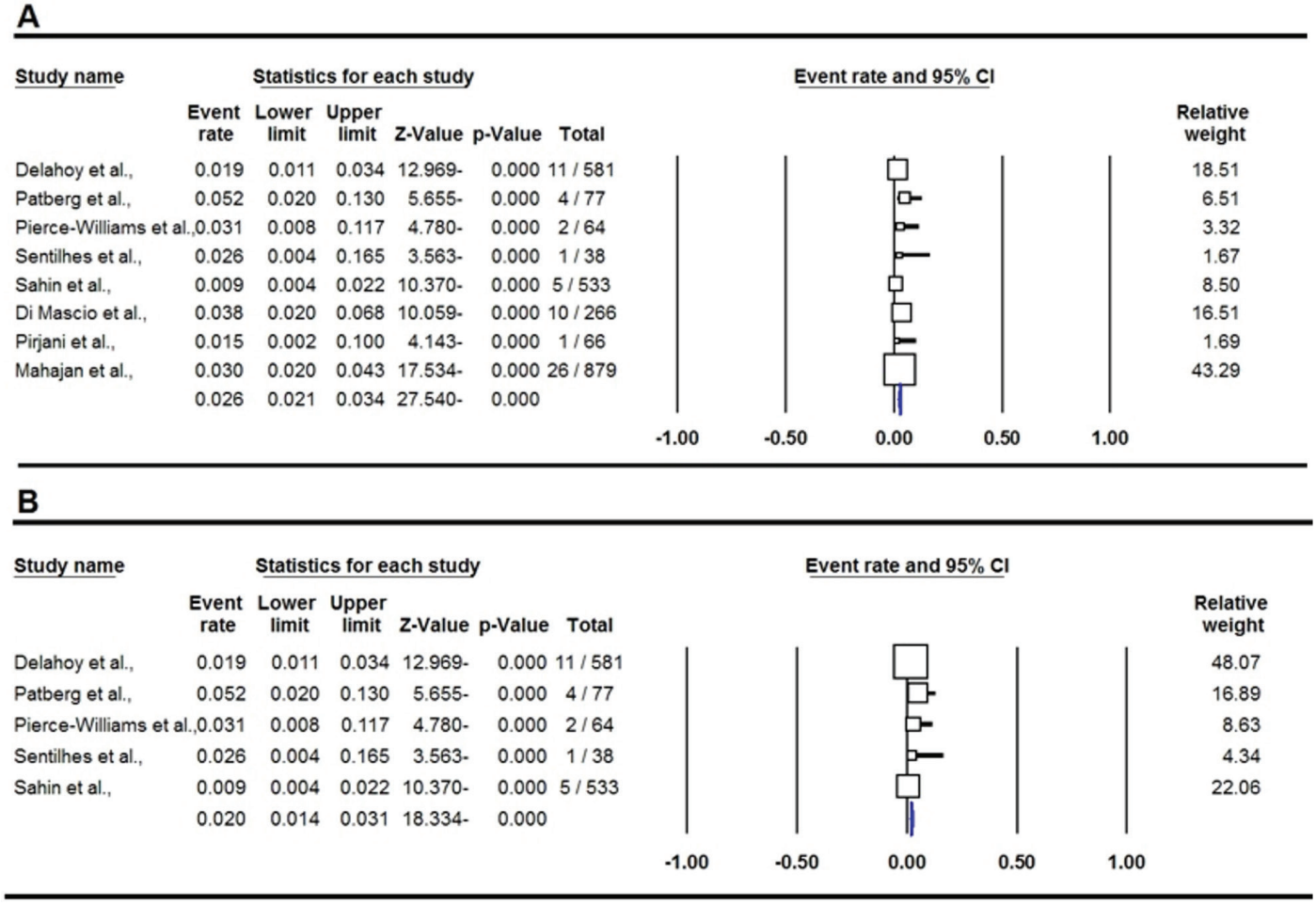

Figure 2. Forest plots of the frequency of IUGR in pregnant women infected with SARS-CoV-2 in the (A) overall and (B) Caucasian populations

IUGR: Intrauterine growth restriction, CI: Confidence interval, SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2

removing each study and then recalculating the corresponding ORs. The data from the sensitivity analysis revealed that none of the studies changed the pooled ORs for IUGR and PPROM, thereby indicating that our combined data are reliable.

Publication Bias

We performed the Begg's and Egger's tests to detect potential publication bias. As shown in Figure 4, the symmetrical funnel plot indicated a significant publication bias for IUGR (PBeggs=0.035; PEggers=0.707) in the overall population but not for PPROM (PBeggs=0.444, PEggers=0.512). Egger's test was performed to provide statistical evidence of the funnel plot (Table 2). The Duval and Tweedie non-parametric "trim-and-fill" method was used to adjust for publication bias. Meta-analyses with and without using the "trim-and-fill" method did not result in different conclusions (Figure 4A).

Discussion

The impact of COVID-19 on pregnancy outcomes remains poorly understood[1,35,36]. The clinical course of COVID-19 in pregnant women has been reported to be similar to that in non-pregnant women[37]. Patberg et al.[14] found in a retrospective study that the frequency of fetal vascular malperfusion abnormalities was significantly higher in pregnant women than in non-pregnant women with COVID-19 by using a multivariable model adjusted for maternal age, ethnicity, preeclampsia, mode of delivery, IUGR/FGR, and oligohydramnios. Placental abnormalities, such as maternal vascular malperfusion, in pregnant women with COVID-19 have also been associated with IUGR[27,38,39]. Studies on pregnant women during previous outbreaks of SARS and MERS observed an increased risk of IUGR. Thus, surveillance of IUGR in women with SARS-CoV-2 infection is recommended because IUGR is often observed in ongoing pregnancies with SARS-CoV-2[37,38].

The incidence of IUGR among pregnant women has been reported to be between 4% and 7%[40]. A recent cohort study involving 4.451 Chinese women found that the incidence of IUGR was 22.4% in women with severe preeclampsia and 18.6% in women with chronic hypertension with superimposed preeclampsia[41]. Diriba et al.[42] performed a meta-analysis on the maternal outcomes of coronavirus infection (i.e., SARS-

Table 1. Characteristics of the studies included in this meta-analysis

First author	City (country)	Ethnicity	Pregnancies or neonate	Number of reports
IUGR				
Delahoy et al.[13]	13 States (USA)	Caucasian	581	11
Patberg et al.[14]	New York (USA)	Caucasian	77	4
Pierce-Williams et al.[15]	Pennsylvania (USA)	Caucasian	64	2
Sentilhes et al.[16]	Strasbourg (France)	Caucasian	38	1
Sahin et al.[17]	Ankara (Turkey)	Caucasian	533	5
Di Mascio et al.[18]	WAPM	Mixed	266	10
Pirjani et al.[19]	Babol (Iran)	West Asian	66	1
Mahajan et al.[20]	Mumbai (India)	South Asia	879	26
PPROM				
Pierce-Williams et al.[15]	Pennsylvania (USA)	Caucasian	64	1
Shanes et al.[27]	USA	Caucasian	16	1
Martnez-Perez et al.[29]	Spain	Caucasian	82	18
Egerup et al.[28]	Copenhagen (Denmark)	Caucasian	28	0
Dávila-Aliaga et al.[30]	Peru	Latin	114	17
Yan et al.[31]	Wuhan (China)	Asian	116	6
Yang et al.[32]	Wuhan (China)	Asian	65	4
Yang et al.[33]	Wuhan (China)	Asian	27	1
Hu et al.[34]	Wuhan (China)	Asian	6	2
Chen et al.[21]	Wuhan (China)	Asian	9	2
Zhu et al.[22]	Wuhan (China)	Asian	9	3
Li et al.[23]	Wuhan (China)	Asian	16	2
Liu et al.[24]	Guangzhou (China)	Asian	13	1
Zhang et al.[26]	Qianjiang (China)	Asian	16	3
Hantoushzadeh et al.[25]	Iran	West Asian	9	1
Mahajan et al.[20]	Mumbai (India)	South Asia	879	29

IUGR: Intrauterine growth restriction, PPROM: Preterm premature rupture of membranes, WAPM: World Association of Perinatal Medicine

Table 2. Summary of the frequencies of IUGR and PPROM among pregnant women with COVID-19

Subgroup	Type of model	Heterogeneity		Odds ratio				Publication bias	
		I^2 (%)	P_H	Frequency	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
IUGR									
Overall	Fixed	34.02	0.157	0.026	0.021-0.034	-27.540	≤0.001	0.035	0.707
Caucasian	Fixed	43.85	0.129	0.020	0.014-0.031	-18.132	≤0.001	0.806	0.590
Asian	Fixed	0.00	0.506	0.029	0.020-0.042	-18.00	≤0.001	1.000	0.718
US	Fixed	36.63	0.206	0.025	0.016-0.040	-14.828	≤0.001	1.000	0.478
PPROM									
Overall	Random	79.27	≤0.001	0.099	0.058-0.164	-7.436	≤0.001	0.444	0.512
Caucasian	Random	74.98	0.007	0.058	0.011-0.248	-3.252	0.001	0.734	0.051
Asian	Random	70.82	≤0.001	0.102	0.056-0.181	-6.411	≤0.001	0.371	0.004
Chinese	Random	47.81	0.053	0.106	0.072-0.155	-9.618	≤0.001	0.465	0.205

IUGR: Intrauterine growth restriction, PPROM: Preterm premature ruptures of membranes, CI: Confidence interval, COVID-19: Coronavirus disease-2019

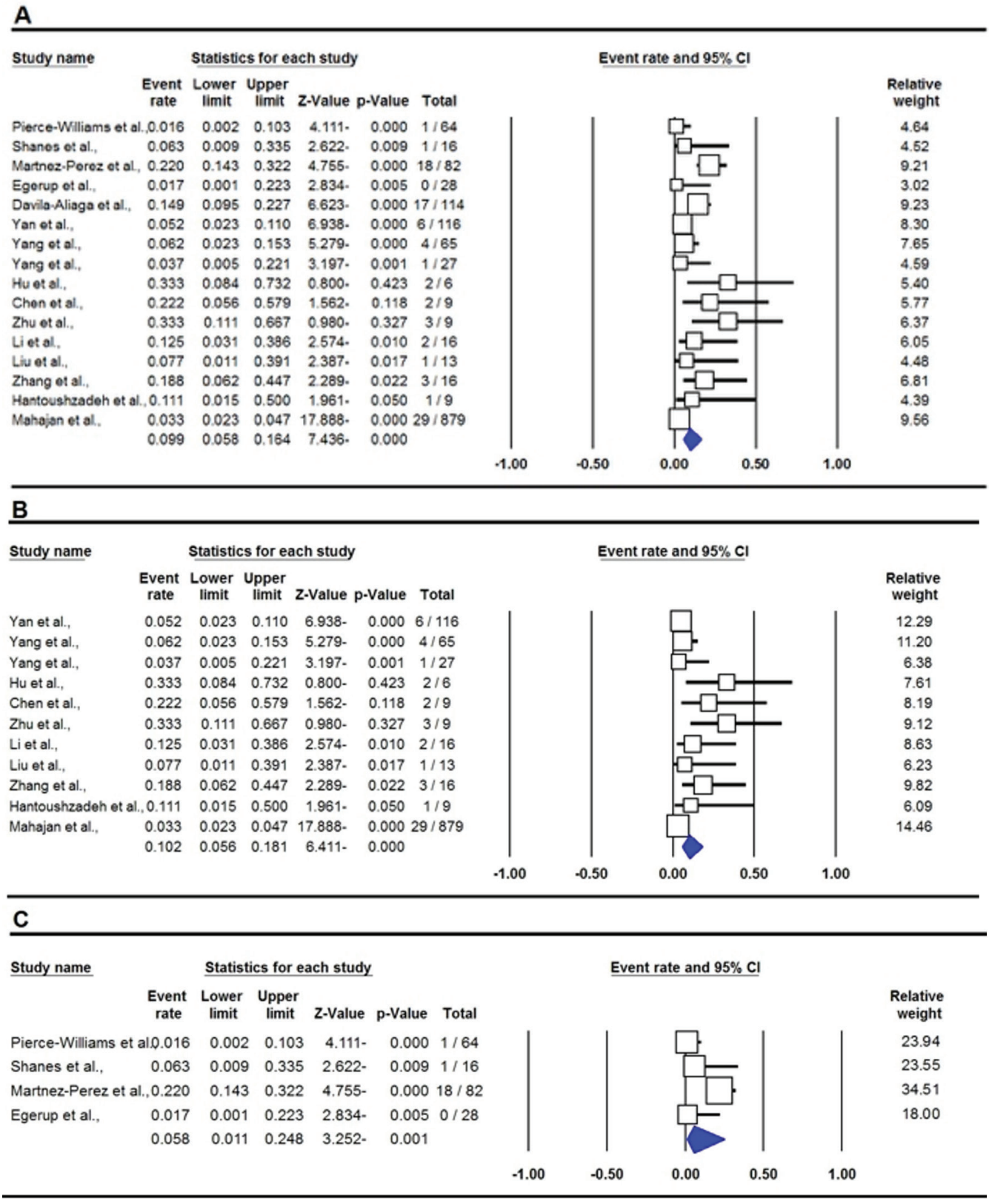

Figure 3. Forest plots of the frequencies of PPROM in pregnant women with SARS-CoV-2 infection in the (A) overall, (B) Asian, and (C) Caucasian populations

PPROM: Preterm premature rupture of the membranes, SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2, CI: Confidence interval

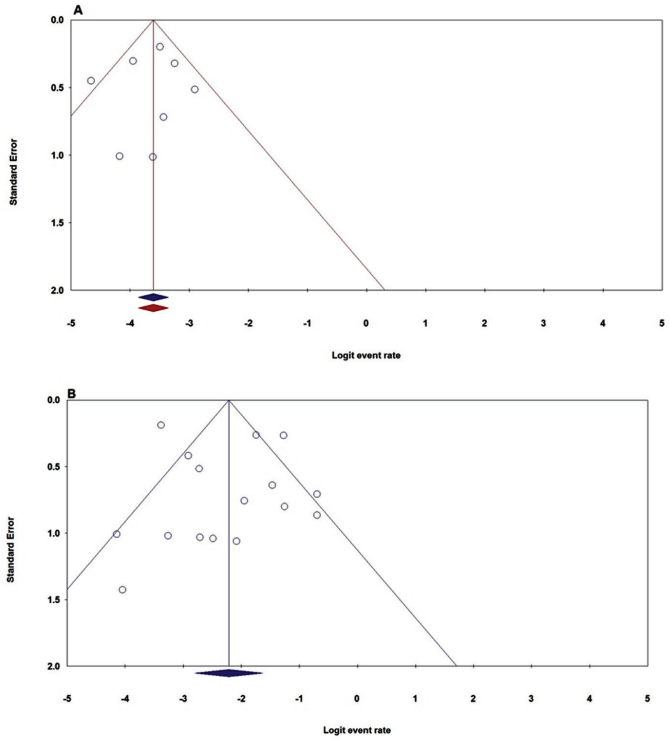

Figure 4. Begg's funnel plot to test for publication bias test on the frequencies of (A) IUGR and (B) PPROM in pregnant women with SARS-CoV-2

PPROM: Preterm premature rupture of the membranes, SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2, IUGR: Intrauterine growth restriction

CoV-2, MERS-CoV, and SARS-CoV) and found that the rates of preterm birth (<37 weeks of gestation), preeclampsia, miscarriage, PPROM, and FGR were14.3%, 5.9%, 14.5%, 9.2%, and 2.8%, respectively. The pooled data revealed that the incidence rates of PPROM and FGR among pregnant women with SARS-CoV-2 infection were 8.9% and 1.2%, respectively. Moreover, the group showed that the incidence rates of PPROM and FGR among pregnant women with SARS-CoV infection were 12.5% and 12.5%, respectively. The current meta-analysis revealed found that the frequency of IUGR in COVID-19 infected pregnant women was 2.6%. This incidence is lower than that determined by others in previously published studies on healthy and infected women[40-42]. Biasucci et al.[11], in a study among 375 Italian pregnant women, demonstrated that COVID-19 was not associated with poorer clinical outcomes and/or with higher rates of preterm birth and IUGR in infected pregnant women compared with non-infected pregnant women. PPROM is defined as the rupture of the fetal membrane prior to the onset of labor at less than 37 weeks of gestation. The incidence of PPROM has been reported to be approximately 3%[40]. However, this incidence varies by ethnicity and region. A retrospective cohort study reported that the incidence of PROM among African-Americans was 29.5%; another study showed that the incidence of PROM in Rochester, NY, USA, was 12.0%[43]. In 2019, Sae-Lin and Wanitpongpan[44] reported that the incidence of PPROM over a 5-year period in Thailand was 2.93%. Our pooled data showed that the frequency of PPROM was 9.9% in COVID-19-infected pregnant women; this figure is substantially higher than that indicated in previous research. Subgroup analysis by ethnicity showed that the respective frequencies of PPROM in COVID-19-infected pregnant women were 10.2% in Asians and 5.8% in Caucasians. This frequency was 10.6% in Chinese women. Rodrigues et al.[45] performed a systematic review including 3.985 COVID-19-infected pregnant women and found that the most frequent obstetric conditions include gestational diabetes (4.5%), PPROM (2.7%), preeclampsia/eclampsia/HELLP syndrome (1.7%), fetal distress (1.1%), gestational hypertension (0.6%), fetal growth restriction (0.4%), placenta previa/placental abruption/placenta accreta (0.4%), and oligohydramnios/polyhydramnios (0.2%). Chi et al.[46], in a meta-analysis including 230 women with COVID-19 and 156 newborns, showed that 8.49% (9/106) of the newborns had PPROM. Della Gatta et al.[47] conducted a review of six studies including 51 pregnant women and found PPROM in at least 9 of 34 patients (26%). Akhtar et al.[48] performed a meta-analysis and determined that the most common maternal/fetal complications included intrauterine/fetal distress (14%) and PPROM (8%). Moreover, the group found that COVID-19 infection in pregnancy leads to increased risk of pregnancy complications, such as preterm birth and PPROM, and may even lead to maternal death in rare cases. Yang et al.[32], in a study based on the Maternal and Child Health Information System of Wuhan, China, showed no significant difference in the incidence of PPROM between the confirmed and free COVID-19 groups. Zhang et al.[26], in a case-control study among pregnant women with and without COVID-19 in Hubei, China, reported no significant difference between pregnant women in terms of gestational diabetes, severe preeclampsia, PPROM, fetal distress, meconium-stained amniotic fluid, premature delivery, neonatal asphyxia, and procedures for severe post-partum bleeding.

Our meta-analysis presents potential limitations. First, all of the included studies were performed among Caucasian and Asian pregnant women with COVID-19. Thus, our pooled data are not generalizable to other ethnicities. Further studies with larger sample sizes in different ethnicities are necessary to confirm our findings. Second, the studies included in the current meta-analysis were published in English and Chinese; thus, a number of potentially significant data published in other languages may have been excluded. Finally, in the current meta-analysis, we could not answer some important questions, such as the extent of asymptomatic or mild infection and the effect on IUGR and PPROM, because of the lack of data in primary studies.

In summary, this meta-analysis showed that the frequencies of IUGR and PPROM in COVID-19-infected pregnant women were 2.6% and 9.9%, respectively. Analyses stratified by

ethnicity revealed that the frequencies of IUGR and PPROM were higher in Asian COVID-19-infected pregnant women than in Caucasian COVID-19-infected women. Given that most studies on COVID-19 included cases with early stages of the disease and that the selected reports were restricted to China, further well-designed studies with larger sample sizes including different populations may be required to obtain more accurate estimates.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: R.B., D.A.S., M.K.Z., Design: R.B., D.A.S., M.K.Z., Data Collection or Processing: M.K.Z., Analysis or Interpretation: S.R.M., H.N., Literature Search: M.N., A.J., S.A.D., F.F., Writing: M.N., R.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Review / Derleme

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Systematic review of the effect of implementing enhanced recovery after surgery on selected attributes of surgical recovery in gynecology

Jinekolojik cerrahide cerrahi iyileşmenin seçilmiş nitelikleri üzerinde cerrahi sonrası hızlandırılmış iyileşme kullanımının etkisinin sistematik derlemesi

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Abstract

This study aimed to systematically review the available literature on enhanced recovery after surgery (ERAS) following gynecologic procedures performed either as an open surgery or as a minimally invasive gynecological surgery (MIGS) in terms of outcomes. This review revealed the results of published literature and assessed the benefits and diverse outcomes of ERAS implementation in patients undergoing MIGS or other gynecologic surgeries. In this review, we sought to examine the efficacy of entire ERAS protocols, faithfully performed, to determine whether they were successful in improving individual attributes of surgical recovery. Electronic databases of PubMed, Cochrane, Web of Science, Scopus, MEDLINE, and ClinicalTrials.gov were systematically searched in January 2021 for relevant studies. Data were extracted from eligible studies including LOS, change in the quality-of-life and recovery over time, postoperative complications including nausea and vomiting, opioid or anesthesia use, hospital cost, patient satisfaction, postoperative pain, and readmission rate as outcomes. Many of the included studies reported a significant reduction in the LOS as well as in readmission rates, hospital cost, and occurrence of nausea and vomiting postoperatively. Moreover, a clinically significant increase was noted in patient satisfaction in studies that have used tools that measure patient satisfaction. No studies have reported a significant increase in the overall quality of recovery using appropriately validated tools. Following ERAS implementation, patients' postoperative rehabilitation, including postoperative discomfort, readmission rates, and satisfaction, showed a clinically significant improvement.

Keywords: ERAS, enhanced recovery after surgery, ERAS protocols, recovery from surgery

Öz

Açık veya minimal invaziv jinekolojik cerrahi (MIGS) ile gerçekleştirilen jinekolojik prosedürlerin ardından "cerrahi sonrası hızlandırılmış iyileşme" (ERAS) hakkında mevcut literatürü farklı sonuçlar açısından sistematik olarak gözden geçirmeyi amaçladık. Yayınlanmış literatürü gözler önüne serdik ve MIGS veya diğer jinekolojik cerrahi geçiren hastalarda ERAS uygulamasının faydalarını ve çeşitli sonuçlarını değerlendirdik. Cerrahi iyileşmenin bireysel özelliklerini iyileştirmede başarılı olup olmadıklarını belirlemek için, sadakatle uygulanan tüm ERAS protokollerinin etkinliğini incelemeye çalıştık. Güvenilir çalışmalar için Ocak 2021'de PubMed, Cochrane, Web of Science, Scopus, MEDLINE ve ClinicalTrials.gov'da sistematik olarak arama yaptık. Hastanede kalış süresi, yaşam kalitesindeki değişiklik ve zamanla iyileşme, bulantı ve kusma dahil postoperatif komplikasyonlar, opioid veya anestezi kullanımı, hastane maliyeti, hasta memnuniyeti, postoperatif ağrı ve yeniden hastaneye kabul oranı gibi veriler, bu değerlendirilen sonlanımları içeren uygun çalışmalardan elde edildi. Dahil edilen birçok çalışma, ameliyat sonrası hastanede kalış süresinin yanı sıra yeniden kabul oranlarında, hastane maliyetinde ve ameliyat sonrası bulantı ve kusmada önemli bir azalma olduğunu bildirdi. Ayrıca, hasta memnuniyetini ölçmek için araçlar kullanan çalışmalarda hasta memnuniyetinde klinik olarak anlamlı bir artış olduğu görüldü. Hiçbir çalışma, uygun şekilde doğrulanmış araçlar kullanarak "toplam iyileşme kalitesinde" anlamlı bir artış bildirmedi. ERAS uygulamasının ardından, ameliyat sonrası rahatsızlık, yeniden kabul oranı ve memnuniyet dahil olmak üzere hastaların ameliyat sonrası rehabilitasyonunda klinik olarak anlamlı bir iyileşme gösterildi.

Anahtar Kelimeler: ERAS, ameliyat sonrası hızlandırılmış iyileşme, ERAS protokolleri, ameliyattan iyileşme

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Introduction

The concept of "enhanced recovery after surgery (ERAS)" was first studied in colorectal surgeries by Kehlet in the 1990s as a bundled pathway to accelerate recovery after surgery[1]. ERAS is a systematic approach to the entire perioperative period aiming to minimize surgical trauma, perioperative stress, and recovery time and maintain postoperative physical function[2,3]. In addition, ERAS protocols can improve mobilization after surgery[4]. These benefits ideally will result in reduced length of hospital stay (LOS), complications, and hospital costs[5-7].

ERAS protocols have now been implemented successfully in various surgical specialties, including gynecology[8]. The ERAS society recommends specific protocols in this specialty, which range from protocols for simple hysterectomy to complex cytoreductive cancer surgeries[9,10]. This society provides guidelines for the preoperative, intraoperative, and postoperative management of patients undergoing gynecological surgery and other types of surgery[11,12]. The ERAS protocols presented by ERAS society for preoperative patient preparation include multiple items such as educating and informing the patient extensively about the planned surgical and anesthetic procedures. Preoperative education and counseling may result in decreased anxiety and increased patient satisfaction, which in turn improve fatigue and promote early discharge[13,14]. Some of the other basic tools employed for preoperative preparation include avoidance of bowel preparation, minimization of preoperative fasting, prevention of surgical site infection, and provision of venous thromboembolism prophylaxis, perioperative nutrition, preoperative laxatives, and opioid-sparing multimodal postoperative analgesia[15].

The ERAS multimodal pain management program is considered a fundamental component in all ERAS protocols, as it can lower opioid consumption and its associated side effects such as sedation, urinary retention, constipation, and poor quality of recovery. This program involves preemptive administration of non-opioid analgesic and other medications such as gabapentin and acetaminophen, along with non-steroidal anti-inflammatory agents or cyclo-oxygenase 2 inhibitors[16-18]. These combinations of preemptive medications were reported to ease postoperative pain and postoperative nausea and vomiting (PONV). Therefore, the consumption of both antiemetic and analgesic drugs may be significantly reduced in patients undergoing these perioperative protocols[16].

Evidence-based guidelines for postoperative care in gynecologic oncology are also available from the ERAS society, including previously mentioned postoperative analgesia, postoperative control of glucose, prevention of postoperative ileus, postoperative thromboembolism prophylaxis, avoidance of peritoneal drainage, early mobilization, and provision of urinary drainage[12]. ERAS protocols and their associated outcomes have been widely studied after exploratory laparotomy in gynecologic surgery and gynecologic oncology. However, relatively little data address ERAS use in benign gynecology and minimally invasive gynecologic surgery (MIGS)[19-22].

MIGS is defined as the use of less invasive techniques such as hysteroscopy or laparoscopy. It requires fewer, smaller incisions (or no incisions at all) instead of one large incision, as is common in laparotomy. With the increased interest in MIGS, including hysterectomy, in the last decade, strategies to further improve outcomes are greatly required[23]. Several studies have recently addressed the effect of ERAS protocols on outcomes of MIGS. In 2016, Michener et al.[23] analyzed some ERAS protocols implemented in patients with gynecologic cancer undergoing gynecologic surgery. Their retrospective case-control study showed that the LOS was decreased significantly in the ERAS group compared with the historical control group. The frequency of using narcotics (measured in morphine equivalents) also decreased in the ERAS group, but the pain scores were not significantly different between the two groups. In 2020, Lee et al.[22] revealed that ERAS protocol adherence by women undergoing MIS for malignant and benign indications did not diminish the median LOS but reduced opioid consumption, hospital costs, and intravenous fluid use.

Given the relative abundance of recently published studies that have analyzed the effect of ERAS protocols on benign gynecology and MIGS, this review aimed to present findings of published studies that have assessed the benefits and diverse outcomes of ERAS implementation in patients undergoing an open surgery or MIGS.

Materials and Methods

Search Strategy and Data Collection

Electronic databases of PubMed, Cochrane, Web of Science, Scopus, and MEDLINE were systematically searched for all studies up to February 1, 2021. Any published results from ongoing studies using the ongoing trials registry of the US National Institutes of Health (http://www.clinicaltrials.gov) were also searched. The literature search was conducted using the following search key terms: (ERAS OR "ERAS" OR "enhanced recovery" OR "enhanced recovery pathway" OR ERP OR "fast-track" OR "fast-tract surgery") AND (MIGS OR "MIGS" OR laparoscopy OR "laparoscopic surgery" OR "robotic surgery" OR "robotic minimally invasive" OR "minimally invasive surgery" OR MIS). The search was limited to gynecology. A preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart of the study selection process is shown in Figure 1.

The endnote software was used to remove duplicated studies, and all retrieved citations were screened for eligibility by screening their titles and abstracts first and then their full texts. Studies that matched the selection criteria were then included in the study. References of the included studies were also screened manually for additional relevant studies.

Selection Criteria

This study included randomized controlled trials (RCTs), cohort studies, and case-control studies that focused on the implementation of ERAS protocols in gynecologic surgeries,

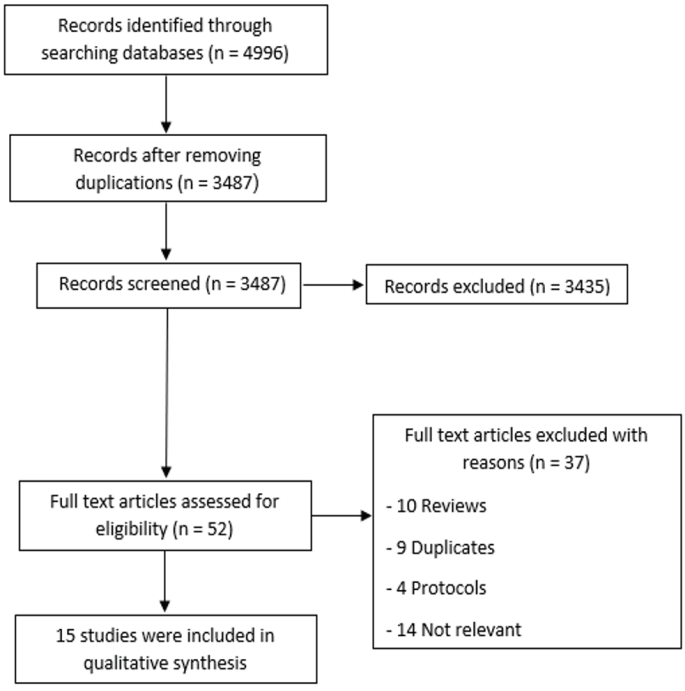

Figure 1. PRISMA flow diagram

PRISMA: Preferred reporting items for systematic reviews and meta-analyses

including oncology, benign gynecology, and MIGS. No restrictions for age, sex, site, or publication date were applied. Animal studies, non-English studies, thesis, reviews, and studies where the full text could not be obtained were excluded.

Data Extraction

Data related to the following were extracted: 1) summary of the included studies and baseline characteristics of their enrolled population including study design, number of patients and samples in each group, age, study outcomes, surgery type, rehabilitation protocol, and conclusion; 2) outcomes including LOS, change in the quality-of-life (QoL) and recovery over time, postoperative complications including nausea and vomiting, opioid or anesthesia use, hospital cost, patient satisfaction, postoperative pain, readmission rate, and ERAS pathway component; and 3) quality assessment questions and domains.

Quality Assessment

The quality of the included RCTs was assessed using Cochrane's risk-of-bias tool (version 1). This tool is found in Chapter 8.5 of the Cochrane Handbook of Systematic Reviews of Interventions 5.1.0. This tool consists of the following domains: sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other bias. Author judgments fell into three categories, including a low, unclear, or high risk of bias for each domain.

The quality of the included cohort and case-control studies was assessed using the National Heart, Lung, and Blood Institute quality assessment tools[24]. These tools are composed of validated questions assessing the risk of bias and confounders. These questions were answered by "yes," "no," "not applicable," "cannot be determined," or "not reported." Finally, each study was given a score to guide the overall quality as "good," "fair," or "poor."

Results

Literature Search

The initial search yielded 4,996 articles from all searched databases. Of these 4,996 articles, 1,509 articles were excluded because of duplications, and titles and abstracts of 3,487 articles were screened. Moreover, 3,435 were excluded as they did not meet the inclusion criteria. The remaining 52 articles underwent full-text screening, of which 37 were excluded and 15 were finally included in the systematic review (PRISMA flow diagram; Figure 1).

Quality Assessment of the Included Studies

The overall quality of the included RCTs was high according to the Cochrane risk-of-bias tool[25-31]. According to the NIH quality assessment tool for observational cohort studies, only one study[32] was graded as good, and the remaining seven studies[22,33-38] had fair quality. One case-control study[39] had fair quality according to the NIH quality assessment tool for observational case-control studies. Full details of the quality assessments can be found in Supplementary Tables S1-3.

Patients and Article Characteristics

From the 15 included articles, eight have reported the LOS, readmission rate, and postoperative pain as outcomes. Five studies have reported the QoL and recovery score, opioid and anesthesia use, and patient satisfaction. Only two studies have reported hospital costs. Table 1 summarizes the characteristics of the included studies and outcomes reported after gynecological procedures.

Outcome Measurements

In this study, the prespecified outcomes that were used as the basis to assess the importance of ERAS implementation were as follows: LOS, change in the QoL and recovery score over time, and postoperative attributes including nausea and vomiting, opioid or anesthesia use, hospital cost, patient satisfaction, postoperative pain, and readmission rate.

LOS

In this study, LOS following the ERAS protocol for surgical intervention was considered as one of the main outcomes that determined the effect of ERAS on patients after surgeries. The ERAS approach may lead to substantial decreases in postoperative pain and LOS and faster return to baseline functioning after laparotomy in various surgical fields[25,26]. Dickson et al.[33]

Table 1. Summary and baseline characteristics of the included studies

Study ID	Study design	Number of patients	Study arm, number	Age (years), Mean ± SD	Study outcomes	Surgery	Rehab protocol	Conclusion
Dickson et al. 2012[33]	Retrospective review of consecutive cases, before and after design	366	First period: control group =86, experimental group =96, second period: control group =90, experimental group =94	First period: control group =45.75±6.5, experimental group =45±9.3 Second period: control group =45±9.5, experimental group =45±9.3	Length of stay, estimated blood loss, duration of surgery, surgical complications	Total abdominal hysterectomy	Rapid-recovery program	Introducing a rapid-recovery program was associated with shorter hospitalization and did not appear to compromise surgical outcome.
Ferraioli et al. 2020[34]	Observational retrospective study	92	Surgery group, 92	61.6±8.17	EVAN-G score	Robotic-assisted or laparoscopic surgery	ERAS	"In this study, we showed a high patient satisfaction with the ERAS program. When comparing length of stay and complications, neither extended length of stay nor development of complications after minimally invasive surgery impacted patient satisfaction."
Frumovitz et al. 2020[26]	Randomized, open-labeled phase 3, non-inferiority trial	631	Open surgery group =312, MIS group =319	Open surgery group =45.6±10.4, MIS group =45.4±1·4	Quality-of-life	Open or minimally invasive radical hysterectomy	-	"Women with early-stage cervical cancer had similar postoperative quality-of-life 6 weeks after surgery and beyond regardless of whether they had open or minimally invasive radical hysterectomy."
Kanno et al. 2019[35]	Retrospective, single-institution study	109	Surgery group =109	43±12.1	Surgical outcomes	Radical hysterectomy	-	"In this retrospective study, MIRH with a no-touch isolation technique for stage IA to IB1 cervical cancer was a safe approach in terms of oncological outcomes. However, every surgeon who treats early-stage cervical cancer should inform each patient of the results of the LACC trial because it has an exceedingly high impact."
Kroon et al. 2010[27]	Prospective, randomized controlled study	53	Total intravenous anesthesia group =27, patient-controlled analgesia =26	Total intravenous anesthesia group =47±5.75, Patient-controlled analgesia =46±5	PONV, bowel function, length of stay, pain, surgical complications	Fast-track hysterectomy	TIVA, patient-controlled analgesia (PCA)	"The TIVA peri- and postoperative care was an advantage over PCA in most respects."

de Lapasse et al. 2008[38]	Prospective feasibility study	35	Surgery group =35	Surgery group =43.0±12.1	Length of stay, patient satisfaction	Total laparoscopic hysterectomy	Anesthetic protocol	"Our protocol for analgesia, anesthesia, and early discharge (24 hours after surgery) may be safely proposed after total LH in selected patients. Satisfaction rate of patients on postoperative days 7 and 30 was very high."
Lee et al. 2020[22]	Cohort study	144	Laparoscopy group =74, open surgery group =70	Laparoscopy group = 54±10, open surgery group = 49±15.5	Self-reported satisfaction questionnaire	Laparotomy	ERAS	"The two groups were in general equally able to achieve most of the milestones despite differences in symptoms such as pain, nausea and confidence in mobilizing and going home. Preoperative education can empower patients. There is a high level of patient satisfaction in both groups."
Ottesen et al. 2002[37]	Prospective descriptive study	41	Surgery group =41	-	Length of stay, patient satisfaction	Vaginal prolapse surgery	Multimodal rehabilitation model	"The need for postoperative hospitalization was median 24 hr after vaginal surgery in a fast-track setting, independently of the complexity of the procedure performed. Short term success rate, satisfaction rates, and acceptability were all excellent. Follow-up has been established to evaluate long-term success rates and recurrence."
Pauls et al. 2015[28]	Randomized double-blinded placebo-controlled trial	63	Placebo group =36, dexamethasone group =27	Placebo group =62.0±9.6; dexamethasone group =63.2±8.3	Quality of recovery, postoperative nausea and vomiting, pain, voiding function	Vaginal reconstructive surgery for pelvic organ prolapses	-	"Use of dexamethasone prior to vaginal reconstructive surgery was associated with less nausea/vomiting and need for antiemetics, as well as greater success with voiding trials. Furthermore, QOR was enhanced, suggesting use of dexamethasone should be considered for these patients."
Peters et al. 2020[36]	Retrospective cohort study	410	Conventional perioperative care, 214; enhanced recovery after surgery, 196	Conventional perioperative care =33.6±10.2, enhanced recovery after surgery =35.1±11.3	Postoperative nausea and vomiting	Non-hysterectomy gynecologic procedures	ERAS	"Enhanced recovery after surgery implementation resulted in increased same-day discharge rates and improved perioperative outcomes without affecting 30-day morbidity in women undergoing laparoscopic minimally invasive non-hysterectomy gynecologic procedures."

Ravndal and Vandrevala. 2016[29]	Randomized double-blinded placebo-controlled trial	24	Intervention group, 12; control group, 12	-	Pain	Laparoscopic surgery	Enhanced recovery program	"Preemptive local anesthetics in the trocar areas are shown to be beneficial in laparoscopic gynecologic surgery within an enhanced recovery program. Movement-evoked pain is far more intense than pain at rest."
Weston et al. 2020[32]	Retrospective study	226	Pre-ERAS group =99, post-ERAS group =127	Pre-ERAS group =58.83±11.92, post-ERAS group =58.25±12.88	Opioid use, pain	Minimally invasive (straight stick laparoscopic, single-incision laparoscopic, or robotic-assisted) hysterectomy	MIS-ERAS protocol	"Enhanced recovery after minimally invasive surgery protocol implementation is an effective means to reduce opioid use, both in the intraoperative and postoperative phases of care among gynecologic oncology patients undergoing minimally invasive hysterectomy."
Wodlin et al. 2011[30]	Secondary analysis from an open multicenter, prospective randomized controlled trial	162	General anesthesia group =82, spinal anesthesia =80	-	Pain, postoperative nausea and vomiting, drowsiness, fatigue, postoperative pruritus	Abdominal hysterectomy	Fast-track program	"Spinal anesthesia with intrathecal morphine carries advantages regarding postoperative symptoms and recovery following fast-track abdominal hysterectomy."
Xiromeritis et al. 2011[31]	Prospective randomized trial	92	Intervention group =47, control group =45	Intervention group =35.7±5.7, control group =33.4±4.7	Pain, length of stay, bowel function	Myomectomy	Multimodal analgesic protocol	"In the setting of minimally invasive myomectomy, the use of a multimodal analgesic protocol improved postoperative recovery, resulting in (an) earlier hospital discharge."
Yoong et al. 2014[39]	Case-control study	100	Surgery group =50, control group =50	Surgery group =51±4.25, control group =49±4	Length of stay, pain, patient satisfaction, cost	Vaginal hysterectomy	ERAS	"The ERAS program in benign VH reduces length of stay by 51.6% and enables more women to be discharged within 24 hours, with no increase in patient readmissions rates."

ID: Identification, SD: Standard deviation, EVAN-G: Evaluation du Vécu de l'Anésthésie Générale, ERAS: Enhanced recovery after surgery, MIRH: Minimally invasive radical hysterectomy, LACC: Laparoscopic approach to cervical cancer, TIVA: Total intravenous anesthesia, PCA: Patient-controlled analgesia, LH: Laparoscopic hysterectomy, QOR: Quality of recovery, ERP: Enhanced recovery protocol, MIS-ERAS: Minimally invasive surgery-enhanced recovery after surgery, VH: Vaginal hysterectomy

found that the median LOS shortened dramatically from 3 days before the implementation to 1 day after the implementation of their ERAS protocol (Rapid-recovery protocol) (p<0.001). In their study of 35 patients, de Lapasse et al.[38] found that 34 (97.1%) patients were discharged the day after surgery and only one patient was not discharged on the surgeon's instructions because of technical difficulties during the procedure. Ottesen et al.[37] reported that postoperative LOS was also 1 day for all patients, except for 3 (7.3%) patients who were discharged later than 48 h. Peters et al.[36] reported that the ERAS implementation significantly increased the same-day discharge rate by 9.4%. Clinically, the ERAS protocol remained effective, with 96.9% of the patients discharged on postoperative day 0 (p<0.005) after excluding those planned postoperative admissions for medical conditions not related to the surgery[36]. Yoong et al.[39] found that after ERAS implementation, the median LOS was reduced by 51.6% (22.0 vs 45.5 h; p<0.01), and the proportion of patients discharged within 24 h was increased by fivefold (78.0 vs 15.6%; p<0.05).

Table S1. Quality assessment of the cohort studies by NIH tool

Domains	Weston 2020	Dickson 2012	Ferraioli 2010	Kanno 2019	Peters 2020	Lee 2018	Ottesen 2002	Lapasse 2008
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA	NR	NA	NR	NR
4. Were all the subjects selected or recruited from the same or similar populations? Were inclusion and exclusion criteria for being in the study pre-specified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	Yes	Yes	NR	No	Yes	NR	NR	NR
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
7. Was the time frame sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	NA	NA	NA	NA	NA	NA	NA	NA
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	No	Yes	No	NR	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	NA	NA	NA	NA	NA	NA	NA	NA
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	No	No	No	No	Yes	No	No
12. Were the outcome assessors blinded to the exposure status of participants?	No	Yes	No	No	No	No	No	No
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) an outcome(s)?	Yes	No	No	No	No	No	No	No
Total scores (Yes =1, No =0.5, NR & NA & CD =0)	10.5	9.5	8.5	8.5	8.5	9	8	8
Quality rating: good (10-14 point) or fair (7-10 point) or poor (0-7 points)	**Good quality**	**Fair quality**	**Fair quality**	**Fair quality**	**Fair quality**	**Fair quality**	**Fair quality**	**Fair quality**

NA: Not applicable, NR: Not reported

Table S2. Quality assessment of the case control studies by NIH tool

Domains	Yoong 2014
1. Was the research question or objective in this paper clearly stated?	Yes
2. Was the study population clearly specified and defined?	Yes
3. Was the participation rate of eligible persons at least 50%?	NR
4. Were all the subjects selected or recruited from the same or similar populations? Were inclusion and exclusion criteria for being in the study pre-specified and applied uniformly to all participants?	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	NR
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes
7. Was the time frame sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	NA
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes
10. Was the exposure(s) assessed more than once over time?	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	No
12. Were the outcome assessors blinded to the exposure status of participants?	NR
13. Was loss to follow-up after baseline 20% or less?	NR
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) an outcome(s)?	No
Total scores (Yes =1, No =0.5, NR & NA & CD =0)	8
Quality rating: good (14-13 point) or fair (8-12 point) or poor (7-0 points)	Fair quality

NA: Not applicable, NR: Not reported

Change in the QoL and Quality of Recovery Over Time

Although subjective, several tools have been developed to measure the QoL and recovery over time elements of postoperative care. Among the included studies, only one measured these attributes and did so using the Functional Assessment of Cancer Therapy tool for Cervical Cancer (FACT-Cx). Frumovitz et al.[26] found no differences in the mean QoL score based on FACT-Cx scores between the non-ERAS and ERAS groups.

Postoperative Attributes

Postoperative complications can be prevalent in the field of open surgery. Dickson et al.[33] reported no clinically significant differences between the ERAS group and the control group regarding complications. Pauls et al.[28] reported that the rates of PONV were not significantly different between the two groups. However, Ferraioli et al.[34] found that 50 (54.3%) patients did not experience any PONV in the ERAS group and 14 (15.2%) patients and 15 (16.3%) patients experienced mild and slight nausea, respectively. Ottesen et al.[37] reported minimal complications after implementation of their ERAS protocol (named "fast track" vaginal surgery) with urinary retention exceeding 450 mL and urinary tract infection (12.2% and 9.8%, respectively) as the most frequent complications. Peters et al.[36] reported that PONV was twice as common in the conventional group than in the ERAS group.

Opioid or Anesthesia Use

Many studies have discussed the use of opioids or anesthesia perioperatively. Dickson et al.[33] reported that local anesthesia improved pain in the experimental group by 83%, reflecting increased use of rapid-recovery modalities ($p<0.001$). Wodlin et al.[30] reported that women who had undergone hysterectomy under spinal anesthesia with intrathecal morphine experienced significantly less postoperative discomfort compared with those who have undergone surgery under general anesthesia. However, PONV were reported equally in the two groups; vomiting significantly more often occurred during the first day after surgery in the spinal anesthesia group.

Hospital Cost

Hospital cost can be difficult to measure secondary to variables such as area and comorbidities, which can be difficult to control for. However, a general cost reduction associated with ERAS implementation was found, likely secondary to decrease LOS. Chapman et al.[25] observed that the average total hospital costs were reduced by 12% in the ERAS group ($13,771) compared with $15,649 ($p=0.01$). Moreover, Modesitt et al.[20] found that hospital costs were significantly decreased by approximately 20% in both ERAS groups that underwent vaginal surgery.

Patient Satisfaction

Despite its subjective nature, many surgeons recognized patient satisfaction as one of the most important intraoperative criteria. Ferraioli et al.[34] found that from a total of 92 patients who received the ERAS protocol, 56 (60.8%) and 30 (32.6%) patients were "very satisfied" and "quite satisfied" with the quality of care received, respectively. In addition, 6 (9.6%) patients were "averagely satisfied," and no patients were dissatisfied with the care provided. Moreover, de Lapasse et al.[38] reported that of 35 women undergoing their ERAS protocol, 34 (97.1%) were satisfied with the procedure and all (100%) patients would

Table S3. Quality assessment of RCTs by Cochrane tool

Domain	Risk of bias	Judgment of the authors
(Frumovitz 2020)		
Random sequence generation (selection bias)	Low risk	"Randomization was done using a computerized minimization program"
Allocation concealment (selection bias)	High risk	"Neither participants nor investigators were masked to treatment allocation"
Blinding of participants and personnel (performance bias)	Low risk	No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding
Blinding of outcome assessment (Detection bias)	High risk	"Open label study, neither participants nor investigators were masked to treatment allocation"
Incomplete outcome data (attrition bias)	Low risk	"The proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate"
Selective reporting (reporting bias)	Unclear risk	Insufficient information to permit judgement of "Yes" or "No".
Other bias	High risk	The study protocol has not been reported.
(Kroon 2010)		
Random sequence generation (selection bias)	Low risk	"The patients were prospectively randomized into two groups using the closed-envelope technique" not stated if the envelope was opaque or not.
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias)	Low risk	No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias)	High risk	Not reported but the study seems to be an open label.
Incomplete outcome data (attrition bias)	Low risk	No loss of follow-up
Selective reporting (reporting bias)	Unclear risk	Insufficient information to permit judgement of "Yes" or "No".
Other bias	High risk	The study protocol has not been reported.
(Pauls 2015)		
Random sequence generation (selection bias)	Low risk	"Randomization schedule was conducted using a computer-generated table into two groups"
Allocation concealment (selection bias)	Low risk	"The patient, physicians, anesthesia personnel, nursing, data collection staff and statistician were all blinded"
Blinding of participants and personnel (performance bias)	Low risk	"The patient, physicians, anesthesia personnel, nursing, data collection staff and statistician were all blinded"
Blinding of outcome assessment (detection bias)	Low risk	"The patient, physicians, anesthesia personnel, nursing, data collection staff and statistician were all blinded"
Incomplete outcome data (attrition bias)	Low risk	All patients that received interventions were included in the analysis
Selective reporting (reporting bias)	Low risk	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way;
Other bias	Low risk	The study appears to be free from any other sources of bias
(Ravndal 2016)		
Random sequence generation (selection bias)	Low risk	"Participants were computer randomized in blocks of 6 by an independent statistician, and the list was delivered to the hospital pharmacy in a sealed envelope."

Allocation concealment (selection bias)	Low risk	"The randomization list contained numbers from 1 to 24 and the allotted local anesthesia or placebo coded groups A and B. The pharmacist then decided which group bupivacaine and saline should represent, and the code was concealed until the end of the study."
Blinding of participants and personnel (performance bias)	Low risk	"The surgeon, the hospital staff, and the participating women were all blinded to what the syringes contained."
Blinding of outcome assessment (detection bias)	Low risk	"The surgeon, the hospital staff, and the participating women were all blinded to what the syringes contained."
Incomplete outcome data (attrition bias)	Low risk	"Only one case was excluded from the analysis"
Selective reporting (reporting bias)	Low risk	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way;
Other Bias	Low risk	The study appears to be free from any other sources of bias
(Wodlin 2011)		
Random sequence generation (selection bias)	Unclear risk	Insufficient information to permit judgement
Allocation concealment (selection bias)	Unclear risk	Insufficient information to permit judgement
Blinding of participants and personnel (performance bias)	High risk	"The study was not blinded"
Blinding of outcome assessment (detection bias)	High risk	"The study was not blinded"
Incomplete outcome data (attrition bias)	High risk	Loss of follow-up was high without any reported reasons
Selective reporting (reporting bias)	Low risk	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way;
Other bias	Low risk	The study appears to be free from any other sources of bias
(Xiromeritis 2011)		
Random sequence generation (selection bias)	Low risk	"Randomization was made using sequentially numbered, opaque, sealed envelopes (SNOSE system)"
Allocation concealment (selection bias)	Low risk	"Neither medical staff nor patients involved in the study were aware of the randomised assignment."
Blinding of participants and personnel (performance bias)	Unclear risk	Insufficient information to permit judgement of "Yes" or "No".
Blinding of outcome assessment (detection bias)	Low risk	"A physician (NP) who was not aware of the assignment conducted postoperative follow-up."
Incomplete outcome data (attrition bias)	Low risk	No loss of follow-up was reported
Selective reporting (reporting bias)	Unclear risk	The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way;
Other bias	Low risk	The study appears to be free from any other sources of bias

RCTs: Randomized controlled trials

recommend it to other patients. Ottesen et al.[37] reported that the patients' satisfaction rate for their protocol ranged from 85.4% to 95.1%.

Postoperative Pain

Ferraioli et al.[34] observed that 29 (31.5%) patients reported not experiencing any postoperative pain in the ERAS group. In addition, 30 (32.6%) and 21 (22.8%) patients in the ERAS group reported slight and moderate pain, respectively, and 7 (7.6%) and 1 (1.1%) patients experienced "a lot" and "enormous" pain, respectively. Lee et al.[22] reported that patients in the laparoscopic group reported better pain control ($p<0.0001$) and nausea control ($p=0.003$) during recovery. This included increased ability to put on their clothes ($p=0.001$) and confidence in mobility ($p<0.0001$) and in going home

(p<0.0001). Weston et al.[32] reported that the pain scores in the ERAS group were lower than those in other patient groups when controlling for oral morphine equivalency (mean 3.6 vs 4.1, p=0.03). Wodlin et al.[30] reported that the most common postoperative symptoms were pain, nausea, vomiting, itching, drowsiness, and fatigue. Abdominal pain, drowsiness, and fatigue occurred significantly less often and with lower intensity in the spinal anesthesia group. Spinal anesthesia was associated with a higher prevalence of postoperative itching[30]. As regards the ERAS in gynecologic surgery and return to bowel function, Xiromeritis et al.[31] reported significantly low visual analog scale scores for postoperative pain, earlier return of bowel peristalsis, and fewer hospitalization hours in the group who received multimodal analgesia.

Readmission Rate

Ferraioli et al.[34] reported that 12 patients required additional postoperative care. This included four patients who were readmitted to the hospital, five were managed on an outpatient basis, and three had a prolonged LOS because of early postoperative complications. de Lapasse et al.[38] reported that 2 (6.7%) patients required admission because of complications. The first patient required hospitalization for a vesicovaginal fistula on day 10 and had to undergo laparoscopic treatment of the fistula. The other patient consulted for hyperthermia on day 4 with a suggestion of cuff cellulitis and was discharged after 2 days of antibiotic treatment. Ottesen et al.[37] reported no readmissions in patients who underwent surgery under ERAS protocol. Peters et al.[36] found that hospital readmission rates (CPC: 2.3% vs ERAS: 3.1%; p<0.584) were comparable.

Discussion

This review revealed and highlighted the importance of ERAS implementations perioperatively in gynecologic surgery. In this study, commonly measured attributes of surgical recovery were reviewed and analyzed using the ERAS protocol from a gynecologic perspective. With regard to the reported outcomes, the ERAS protocol appears to have positive effects on patients both clinically and psychologically. In addition to being the most recent, our review is more comprehensive than previous endeavors and includes cohort and retrospective analysis not covered in previous reviews. The systematic review by Kalogera et al.[21] in 2019 concluded that ERAS implementation in gynecologic surgery represents the best clinical practice and should be adopted across gynecological surgical procedures. Despite the lack of conflict between the results of our review and those of their review, we would not be in favor of such a strong recommendation, which we believe should be reserved for the day when more diverse and high-quality data allow a full meta-analysis of this critical topic. However, we concur that ERAS protocols in gynecologic surgery appear to improve postoperative pain, satisfaction, and decrease LOS in appropriate patients. ERAS protocols have the potential for universal adoption across gynecologic surgeries if further RCTs and high-quality studies continue to report similar results.

As regards opioid use, Weston et al.[32] concluded that the implementation of the ERAS protocol after MIS is an effective method to reduce opioid use. In addition, Wodlin et al.[31] reported that spinal anesthesia appears to reduce the need for opioids postoperatively. They also reported that spinal anesthesia with intrathecal morphine demonstrated favorable effects on postoperative symptoms and recovery following fast-track abdominal hysterectomy[30]. In the setting of minimally invasive myomectomy, Xiromeritis et al. [31] reported that the implementation of a multimodal analgesic protocol improved postoperative recovery, resulting in earlier hospital discharge.

Ljungqvist et al.[40] and Helou et al.[41] concluded that ERAS could enhance postoperative outcomes, satisfaction, and care costs for most patients undergoing gynecologic surgery. However, Helou et al.[41] also stated that some modifications to the current ERAS protocols may benefit specific subgroups of patients, including patients with chronic pelvic pain, opiate dependence, or psychiatric disorders. Wong et al.[42] concluded that with ERAS, minimally invasive gynecologic surgeons could help minimize and manage postoperative pain with less dependence on opioid medications. In his review, Bajsová concluded[43] that the implementation of an ERAS protocol could lead to a reduction in complications of up to 40% and a reduction in hospitalizations of up to 30% and thus reducing the overall costs without increasing the rehospitalization rate.

As regards the audit of surgical practice, Wijk et al.[44] concluded that with ERAS guidelines, surgical practice demonstrates improvements in compliance and clinical outcomes, including LOS.

The strength of this study lies in our comprehensive search of the current literature to obtain the highest and most dependable level of evidence regarding perioperative implementation of the ERAS protocol in gynecologic surgery up to this point. This review included most study designs to ensure a large sample size. However, weaknesses include the moderate overall quality of the included studies and insufficient data for meta-analysis. Further studies with larger sample sizes and longer follow-up are essential to involve more ERAS outcomes.

Conclusion

In conclusion, ERAS implementation showed a clinically significant improvement in patient recovery postoperatively, including postoperative pain, readmission rates, and satisfaction. Further studies are necessary to formulate stronger, broader recommendations regarding the adoption of ERAS protocols across gynecologic surgeries.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: J.P., Design: A.A., Data Collection or Processing: C.C., S.R., Analysis or Interpretation: G.B., K.C., Literature

Search: G.J.M., C.C., A.T., A.K., S.R., G.B., K.C., N.C., H.U., J.P., A.A., K.S., Writing: G.J.M.

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Prenatal diagnosis of diastrophic dysplasia in the second trimester of pregnancy: Two- and three-dimensional ultrasonographic findings

Gebeliğin ikinci trimesterinde diastrofik displazinin prenatal tanısı: İki ve üç boyutlu ultrasonografik bulgular

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Abstract

To present a prenatal diagnosis of diastrophic dysplasia in the second trimester of pregnancy using two- (2D) and three-dimensional (3D) ultrasonography. The mother was primigravida and aged 12 years. She underwent the first 2D obstetric ultrasound examination at 27 weeks, showing bilaterally upper and lower limb micromelia, thumb and hallux in bilateral abduction, bilateral talipes equinovarus; hyperlordosis of the lumbar spine, cervical, lumbar, and sacral scoliosis; cervical hyperkyphosis with the misalignment of cervical vertebrae, and straight clavicles. 3D ultrasonography in conventional and HDlive rendering modes confirmed the changes observed in 2D ultrasonography and allowed improved understanding by the parents. At birth, the newborn presented transient respiratory distress and neonatal sepsis. At the time of writing, the child is aged 31 months and under follow-up by the pediatrics department. 3D ultrasound allowed the parents to understand the fetal malformations better, and they received adequate counseling.

Keywords: Diastrophic dwarfism, osteochondrodysplasias, skeletal dysplasia, ultrasonography

Öz

Bu çalışmada, gebeliğin ikinci trimesterinde, iki (2D) ve üç boyutlu (3D) ultrasonografi kullanarak prenatal diastrofik displazi tanısı konan bir hasta sunulmuştur. Anne primigravida idi ve 12 yaşındaydı. İlk 2D obstetrik ultrason muayenesi 27. haftada yapıldı. Fetüste bilateral üst ve alt ekstremitelerde mikromeli saptandı. Her iki baş parmak abdüksiyonda idi. Bilateral talip ekinovarus, lomber hiperlordoz, servikal, lomber ve sakral skolyoz, servikal omurların yanlış hizalanması ile birlikte servikal hiperkifoz ve düz klavikulalar saptandı. Geleneksel ve HDlive oluşturma modlarında 3D ultrasonografi, 2D ultrasonografide gözlemlenen değişiklikleri doğruladı ve durumun ebeveynler tarafından daha iyi anlaşılmasını sağladı. Yenidoğanda geçici solunum sıkıntısı ve neonatal sepsis görüldü. Bu yazı yazıldığı sırada çocuk 31 aylık idi ve pediatri serviside takip ediliyordu. 3D ultrason, ebeveynlerin fetal malformasyonları daha iyi anlamalarını ve yeterli danışmanlık almalarını sağlamıştır.

Anahtar Kelimeler: Diastrofik cücelik, osteokondrodisplaziler, iskelet displazisi, ultrasonografi

Introduction

Diastrophic dysplasia (DD) is a rare skeletal malformation because of an autosomal recessive genetic alteration[1] with an incidence of 1 case per 100,000 live births[2]. This condition was first described in 1960. It is characterized by predominantly rhizomelic micromelia, crooked feet, deformed earlobes, joint contractures, scoliosis, as well as hand changes. Usually, cognitive development has no changes[2,3].

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DD is caused by a mutation in the *SLC26A2* gene, which encodes the sulfate carrier anion. Therefore, the condition is called "DD of the sulfate carrier"[2]. The gene mutation is located on the long arm of chromosome 5q31-q34. Mutations in this gene cause a defect in sulfate transporters of chondrocytes and fibroblasts, which interferes with endochondral ossification and consequently leads to abnormal growth and remodeling of bones and cartilage[4].

The first prenatal diagnosis, by two-dimensional (2D) ultrasound, was performed in 1980. Usually, the condition is diagnosed during the second trimester of pregnancy, but it can be diagnosed sooner with a routine ultrasound from the 11th to the 14th week[3,5,6]. According to a PubMed search using the terms "DD," "diastrophic dwarfism," and "prenatal diagnosis," 13 prenatally diagnosed cases have been published in the English-language literature[2-14]. Sepulveda et al.[9] were the first to describe a case report of prenatal diagnosis of DD using three-dimensional (3D) ultrasonography. Although a detailed 2D ultrasound evaluation can reveal shortening long bones, scoliosis, club feet and fixed, bilateral abduction of the thumbs and toes, 3D ultrasound provides clearer views of the limb anomalies, including the hitchhiker thumbs[7,9,15].

DD is considered a severe but non-lethal skeletal dysplasia[9]. Death rates until early childhood reach 25%, primarily because of airway obstruction and laryngotracheal stenosis[9,16]. The main differential diagnoses are campomelic dysplasia, thanatophoric dysplasia, osteogenesis imperfecta, distal arthrogryposis, Larsen syndrome, multiple pterygium syndrome, Roberts syndrome, Kniest dysplasia, mesomelic dysplasia, chondrodysplasia punctata, and achondrogenesis[7,11,15].

This is the report of a case of prenatal diagnosis of a fetus with DD in which the main results of 2D and 3D ultrasonography are highlighted.

Case Report

The mother was primigravida, aged 12 years, with a late start of prenatal care, at 25 weeks of gestation. She reported no comorbidities before pregnancy and no use of psychoactive substances. During prenatal follow-up, subclinical hypothyroidism was diagnosed. There is no report of parents' consanguinity or family history of skeletal malformations.

The first 2D obstetric ultrasound examination was performed at 27 weeks, showing micromelia of all limbs (Figure 1); bilateral talipes equinovarus; hyperlordosis of the lumbar spine; cervical, lumbar, and sacral scoliosis; cervical hyperkyphosis with misalignment of cervical vertebrae; straight clavicles; normal thoracic appearance (cardiac area to thoracic area ratio: 0.31); chest circumference to waist circumference ratio: 0.77; femoral length to waist circumference ratio: 0.14; dilated cisterna magna; and an estimated fetal weight of 602 g (percentile 0.6). Fetal echocardiography demonstrated no evidence of abnormalities. To confirm the results observed with 2D ultrasonography, 3D ultrasonography was performed in both conventional and HDlive rendering modes, confirming the previous findings and evidencing bilateral abduction of the thumbs and halluces (Figure 2). 3D ultrasound allowed the parents to understand the fetal malformations better, facilitating counseling.

A new ultrasound series was obtained at 32 weeks and 1 day, showing a fetus with an estimated weight of 1198 g (percentile 0.4), chest circumference to waist circumference ratio of 0.67, and femoral length to waist circumference ratio of 0.14. The images evidenced a narrow thorax (heart area to thoracic area ratio: 0.54), hypotelorism, and straight clavicles (Figure 3). The parents refused karyotyping and fetal genetic testing even after psychological, fetal medicine, and geneticist consultant counseling. At 38 weeks of gestation, the ultrasound images demonstrated an estimated fetal weight of 1520 g (percentile zero); normal body and respiratory movements; normal amniotic fluid (largest vertical pocket: 4.0 cm); and umbilical

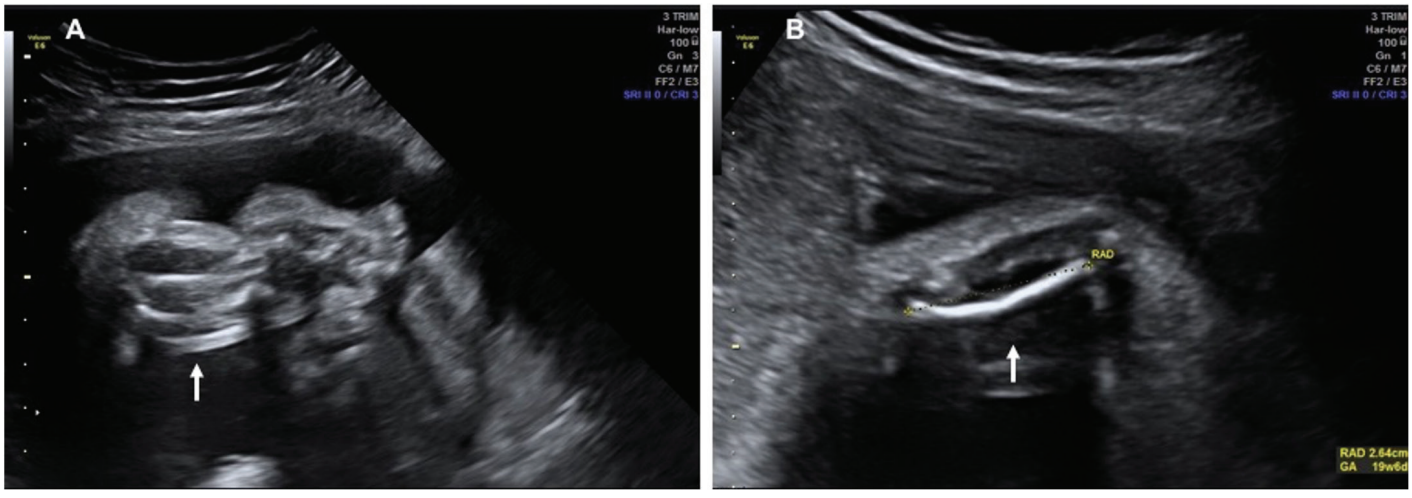

Figure 1. Two-dimensional (2D) ultrasound images of the fetus at 27 weeks of gestation showing lower and upper limbs. (A) Short lower limb (arrow). (B) Short upper limb (arrow)

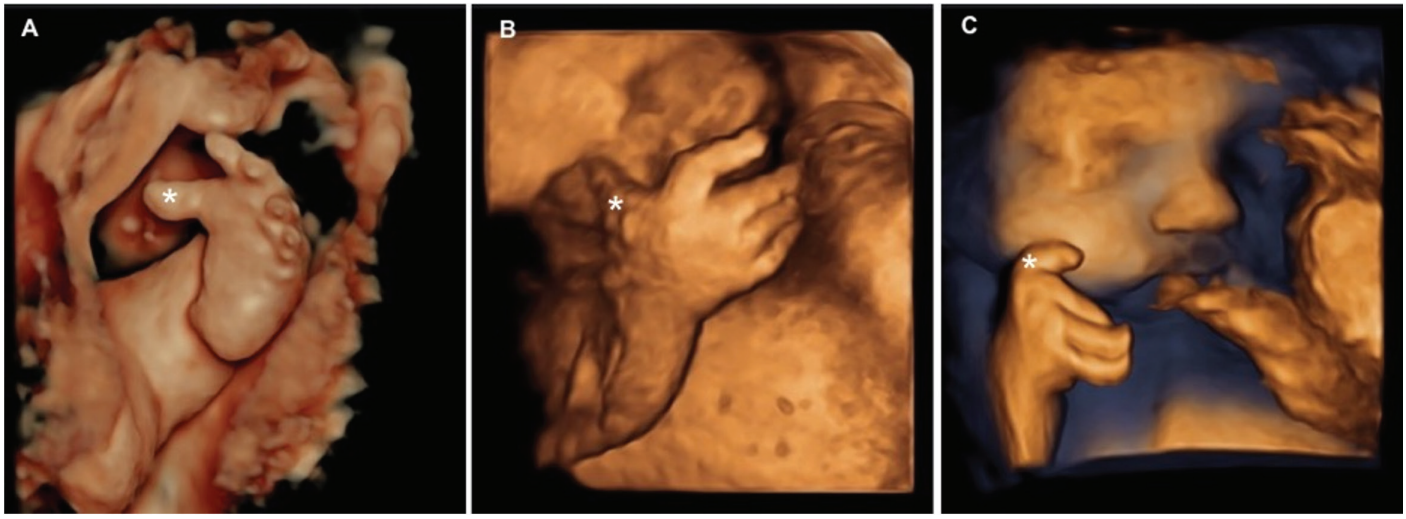

Figure 2. Three-dimensional (3D) ultrasound images of the fetus at 27 weeks of gestation, demonstrating fetal hand and foot. (A) Hallux in abduction (*). (B and C) Thumbs in abduction (*)

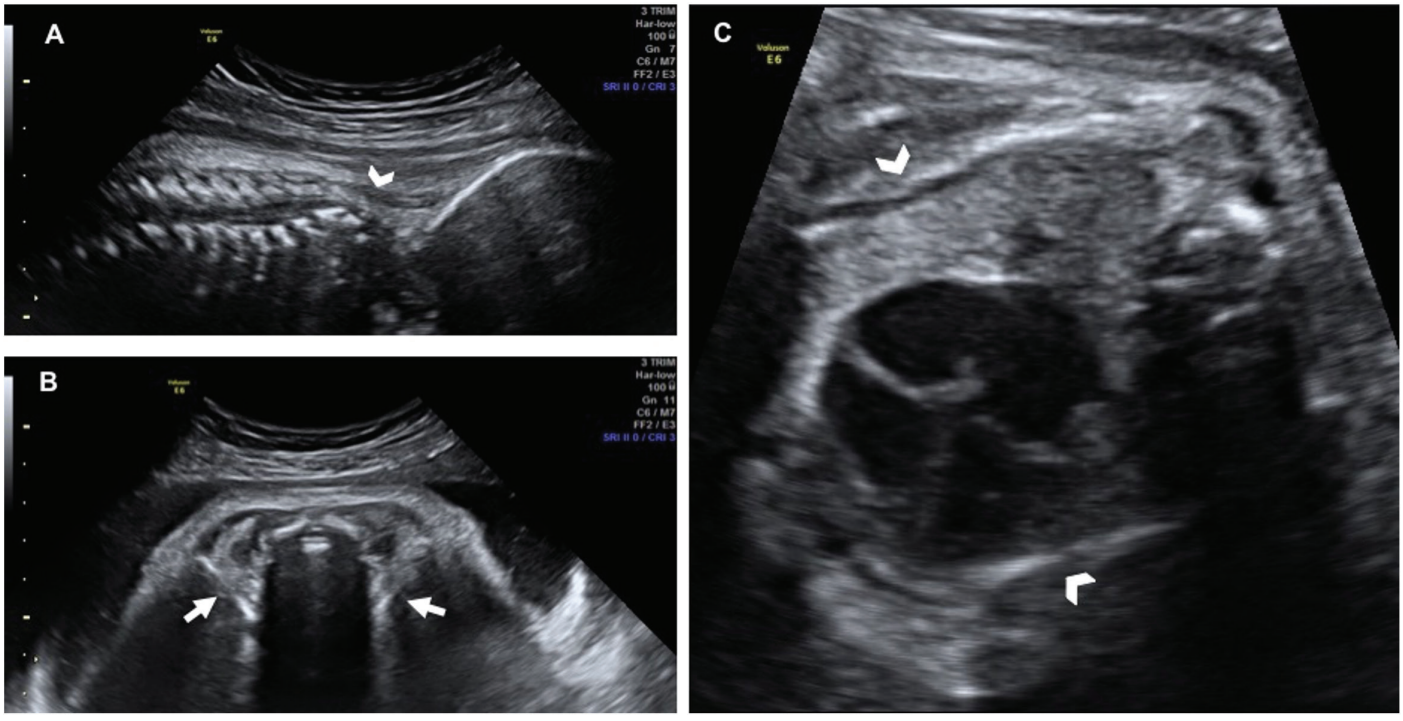

Figure 3. Two-dimensional (2D) ultrasound images of the fetus at 32 weeks of gestation showing abnormalities in fetal spine, clavicles and thorax. (A) Cervical spine misalignment (arrowhead). (B) Straight clavicles (arrow). (C) Thoracic narrowing (arrowhead)

artery Doppler with positive diastolic flow and increased pulsatility index (PI); and middle cerebral artery with PI adequate for gestational age. A cesarean section was performed due to maternal request. As the pregnant woman was an early adolescent, the parents asked for the cesarean section ether, even after medical and psychological counseling regard the benefits of vaginal delivery. The child was born alive, a boy weighing 2415 g, height of 36 cm, head circumference of 32 cm, thoracic circumference of 29 cm, abdominal circumference of 33 cm, and an Apgar score of 8 and 9 at the first and fifth min, respectively.

The newborn evolved with a heart rate of 60 bpm and apnea. After neonatal resuscitation maneuvers, impaired movement was observed because of limb malformations, lowering of the anterior part of the skull, auricular hematoma, long second and third fingers bilaterally, thumbs with persistent pressure, and cone-shaped thorax (Figure 4).

The newborn was referred to the neonatal intensive care unit, where he remained hospitalized for four days, evolving with improvement of the respiratory distress and without complications. Echocardiography was performed, showing a

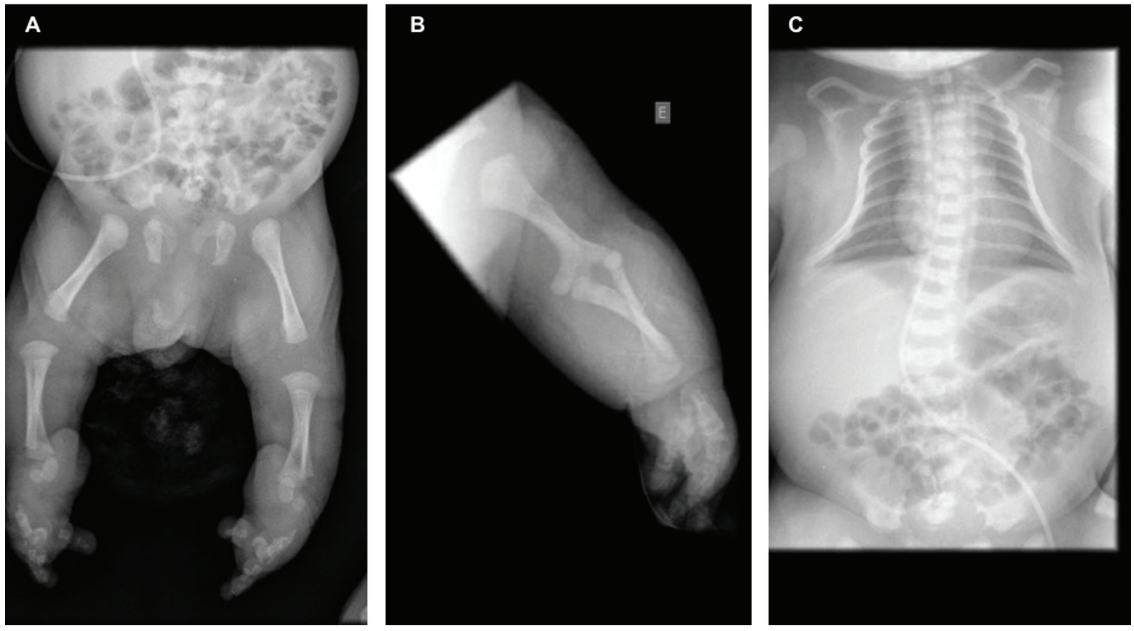

Figure 4. X-ray images of the newborn showing micromelia of lower (A) and upper (B) limbs and thoracic narrowing (C)

pervious foramen ovale. A chest X-ray showed a narrow, bell-shaped thorax. An X-ray of the upper and lower limbs confirmed micromelia. A computed tomography scan of the skull showed no abnormalities. The neonatal heel-prick test showed traces of hemoglobin C. During this period, the respiratory distress improved but the newborn presented physiologic jaundice and neonatal sepsis. The septic condition was solved without complications with antibiotic therapy (ampicillin at 192.3 mg/kg/dose and gentamicin at 4.3 mg/kg/day).

During the outpatient follow-up with a geneticist physician, a diagnostic hypothesis of DD and atelosteogenesis type II was formulated, based on the phenotypic picture. No cytogenetic tests were performed because of the parents' refusal. At the time of writing, the child is aged 31 months and under follow-up by the pediatrics department.

The description of this report was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (Certificate of Presentation for Ethical Assessment: 39343420.7.0000.8667). Both the patient and her legal guardian signed an informed consent form.

Discussion

This case of DD was diagnosed in a very young adolescent, without any risk for the disease, using 2D and 3D ultrasound at the end of the second trimester of pregnancy; most ultrasound results were confirmed in the neonatal period.The interest of our report is based on three facts. First, there is only one case report on the contribution of 3D ultrasound in the diagnosis of DD[9]. Secondly, the diagnosis of DD was performed in a patient without risk for the disease. There are a few cases reported of the diagnosis of DD in a non-risk family[6,7,9,11,15,17]. Thirdly, this case reinforces the importance of a multidisciplinary approach manage of DD.

DD is a generally severe, rare osteochondrodysplasia, first described in 1960 by Lagoy et al[1]. Its prevalence is unknown; however, it is more frequent in Finns (3:100,000), a population in which it is the most common skeletal dysplasia[1]. DD is an autosomal recessive inherited condition, encoded by a gene in the long arm of chromosome 5. Therefore, the risk of being present in another pregnancy of the same mother is 25%[1,9].

DD used to be known as diastrophic dwarfism, and it was confused with achondroplasia. Studies suggest that the biochemical defect of DD is attributed to mutations in the sulfate transporter gene (*SLC26A2*), which impairs the functioning of the sulfate-chloride exchange in the cell membrane and leads to changes in the endochondral calcification process and consequently the abnormal formation of cartilage, interfering with the formation of the skeletal system and growth[2,4,6,10,11].

SLC26A2 gene mutations are responsible for four clinical manifestations of chondrodysplasia: "classic" DD, multiple epiphyseal dysplasia type 4 (MED 4), atelosteogenesis type II (AO-II) and achondroplasia type 1B (ACG-1B). ACG-1B is the most severe form, often lethal either before or shortly after birth. AO-II is a form of chondrodysplasia with clinical and histopathologic manifestations similar to those found in "classic" DD; however, with additional severe phenotypes, it is associated with pulmonary hypoplasia and laryngeal stenosis with death usually during the neonatal period. MED 4 is the mildest form, characterized by joint pain, usually in the hip and knees, deformities in the hands, feet, and knees, and scoliosis[2,6,10]. In the presently reported case, evaluation with a geneticist suggested the hypotheses of DD and OO-II; however, no array comparative genomic hybridization test

was performed for diagnostic confirmation, due to the parent's refusal to perform the tests during the prenatal and postnatal period. We believe that the very young adolescent maternal age and low social and economic level of the legal guardian for the adolescent contributed to the decision.The diagnosis of DD has already been described during the first trimester of pregnancy using 2D ultrasound[3,5]. Adolescent pregnancy and early motherhood continue to be a global public health burden[18]. The experience of multiple adversities, stress, and anxiety during pregnancy in adolescence and throughout the motherhood process could diminish the ability of the young mother to be self-efficacious[19]. In our case report, the very young adolescent maternal age may have influenced the late access of prenatal care and consequently the diagnosis of DD only at the end of the second trimester of pregnancy. During the pregnancy and puerperal period, the patient was supported by a psychology team and social workers.DD is clinically characterized by predominantly rhizomelic micromelia, i.e., long bones, crooked feet (talipes equinovarus), deformed earlobes with a cauliflower-like appearance, joint contractures, scoliosis, hand deformities, and short stature, but normal intelligence[3,12]. Deformities of the hands include brachydactyly and symphalangism of the proximal joints of the second to the fifth finger, as well as thumbs in abduction ("hitchhiker's thumb"), which are considered a pathognomonic sign of DD, although it is not always present[3,12]. To the best of our knowledge, this is the second report on the use of 3D ultrasound in the diagnosis of DD. Sepulveda et al.[9] through 3D ultrasound easily demonstrated the abnormalities of long bones, hitchhiker thumbs and facial anomalies of a fetus with DD. The advantages of 3D over 2D ultrasound could be clinically important, as micrognathia and hitchhiker thumbs could easily be missed on 2D ultrasound[7,9,15]. In the present case, 3D ultrasound in rendering mode allowed a detailed evaluation of the hands and feet, including the abducted halluces and thumbs.

Other deformities that often occur in cases of DD include narrow thorax, tracheomalacia, micrognathia, and cleft palate[2-6]. In this case, the fetus had cervical and lumbar scoliosis, significant micromelia, chest narrowing, bilateral talipes equinovarus, and absence of pulmonary hypoplasia or cleft palate. After birth, rhizomelic limb shortening, bell-shaped thorax, disproportionate skull and face, auricular hematoma, bilateral abducted thumbs, and bilateral talipes equinovarus were confirmed.

The combination of clinical, radiologic, and histopathologic features allows the diagnosis of DD at birth[2]. Prenatal diagnosis is possible via 2D ultrasound; such a diagnosis was first reported in the early 1980s. Most diagnoses of DD were made in fetuses in the second trimester, as in the present report. The presence of micromelia, congenital talipes equinovarus, and finger deformities, especially thumbs in permanent abduction facilitates the recognition of this pathology. Currently, many researchers consider 3D ultrasound as the method of choice for DD diagnosis[2-4]. In this case, 3D ultrasound was useful for documenting the bilaterally abducted thumbs and halluces and the talipes equinovarus, facilitating the understanding of fetal malformations by the parents and thereby allowing better genetic counseling.

The definitive diagnosis of DD is made via histopathologic examination, which demonstrates the progressive destruction of cartilage, disorganization and degeneration of chondrocytes, and irregular myxoid degeneration with replacement by fibrous and even bone connective tissue. It is necessary to conduct differential diagnosis with other micromelias such as achondroplasia, congenital multiple arthrogryposis, and spondyloepiphyseal dysplasia congenita[3].

During the neonatal period, the death rate from DD is high (25%), usually attributed to airway obstruction. Thus, perinatal management involves strict monitoring for respiratory complications because of the risk of restrictive airway obstruction caused by tracheomalacia, progressive cervical kyphoscoliosis, and joint disease, which may be associated with congenital heart defects[2,3]. In the present case, the only respiratory complication described was neonatal respiratory distress, which required two cycles of positive-pressure ventilation after birth. The newborn had no congenital heart disease identified either clinically or by neonatal echocardiography. During outpatient follow-up, he has shown no signs of other serious respiratory complications to date.

The treatment of DD cases comprises physiotherapy and other correlated therapies that help improve mobility[2]. Physiotherapy treatment has been offered for motor improvement and for correcting talipes equinovarus.

Conclusion

In terms of diagnosis and perinatal prognosis, the prenatal diagnosis of DD during the second trimester of pregnancy has fundamental implications. 3D ultrasound, through multiplanar evaluation, may increase the detection of the abnormalities of long bones, hands and facial abnormalities. Moreover, it allows the parents to understand fetal malformations better with adequate counseling.

Ethics

Informed Consent: Both the patient and her legal guardian signed an informed consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.S.L., B.G.F., C.W.L.S., Concept: M.C.P., Design: A.B.P., Data Collection or Processing: H.Y., Analysis or Interpretation: C.G.P., Literature Search: I.B.C.B., Writing: E.A.J.

Conflict of Interest: The authors declare no conflict of interest.

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Localized endometrioid cancer in the abdominal wall with synchronous early-stage endometrial cancer

Batın duvarında erken evre endometrium kanseri ile senkron lokalize endometrioid kanser

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Abstract

Presented herein is the only case in literature with the synchronous development of endometrioid-type endometrium cancer and endometrioid carcinoma originating from the endometriosis foci in a scar tissue. A 44-year-old female patient presented with complaints of abnormal uterine bleeding, swelling at the rectus muscle level in the abdominal wall, and cyclic pain close to the old cesarean section incision scar. Pathological findings of the rectus muscle and endometrial biopsies revealed endometrioid adenocarcinoma (grades 2 and 1, respectively). Positron emission tomography, performed for primary focus investigation, revealed pathologic fluorodeoxyglucose uptake in the uterine cavity and biopsy site, consistent with residual tumor without any pathologic uptake elsewhere. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and systematic pelvic and para-aortic lymphadenectomy, and the residual tumor in the left rectus muscle was excised. The patient was followed up for 3 years. At a postoperative follow-up in the 4th year, no relapsed tumor or metastasis was seen on imaging.
Keywords: Endometriosis, endometrioid adenocarcinoma, scar

Öz

Endometriozis, ovaryan hormon uyarımına yanıt veren, uterus boşluğunun dışına implante olmuş bezler, stroma ve endometriyal dokunun varlığı ile karakterizedir. Endometrioid tip endometrium kanseri ve endometrioid karsinomun skar dokusunda endometriozis odaklarından kaynaklanan senkron gelişimi olan tek olguyu literatürde sunduk. Kırk dört yaşında kadın hasta, anormal uterin kanama, karın duvarında rektus kası seviyesinde şişlik ve eski sezaryen kesi izine yakın siklik ağrı şikayetleri ile başvurdu. Rektus kası patolojik bulguları ve endometriyal biyopsiler endometrioid adenokarsinomunu (sırasıyla; grade 2 ve 1) gösterdi. Birincil odağın araştırılması için gerçekleştirilen pozitron emisyon tomografisi, başka bir yerde herhangi bir patolojik tutulum olmaksızın tümör ile uyumlu olarak uterin kavite ve biyopsi bölgelerinde patolojik florodeoksiglukoz tutulumunu ortaya çıkardı. Hastaya total abdominal histerektomi, bilateral salpingo-ooferektomi, sistematik pelvik ve paraaortik lenfadenektomi yapıldı ve sol rektus kasındaki rezidüel tümör eksize edildi. Hasta üç yıl takip edildi. Postoperatif 4. yılda görüntülemede nükseden tümör veya metastaz görülmedi.
Anahtar Kelimeler: Endometriozis, endometrioid adenokanser, skar

Introduction

Endometriosis is characterized by the presence of endometrial tissue with glands and stroma implanted outside the uterine cavity, which respond to ovarian hormone stimulation[(1)]. The disease affects at least 10% of women with a child-bearing potential in the United States[(2)]. Despite its high prevalence, its etiology remains unclear. Pelvic endometriosis is nowadays a common condition encountered by gynecologists and infertility specialists. Extrapelvic endometriosis in distant sites, such as the urinary bladder, umbilicus, gastrointestinal tract, and thoracic cavity, is a rare condition. Even rarer is scar endometriosis; its pathology is different from other locations of endometriosis. Scar endometriosis occurs due to the iatrogenic implantation of endometrial tissue during uterine procedures and very rarely after non-uterine procedures. The incidence of scar endometriosis after a cesarean section (C-section) has been reported to be 0.03-0.4%[(3)]. The mean time interval between a previous uterine surgery and the diagnosis of abdominal wall endometriosis was found to be 2.3±2.2 years[(4)]. Herein, we report the case of a patient with endometrioid adenocarcinoma

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that arose in a focus of extrapelvic endometriosis. This case report was edited according to the CARE guideline[5].

Case Presentation

A 44-year-old female patient presented to a healthcare institution with swelling at the rectus muscle level in the abdominal wall between the left side of the umbilicus and previous C-section incision scar that progressively enlarged within the last 6 months. The patient also complained of menorrhagia. Incisional biopsy of the patient's rectus muscle and endometrial biopsy were performed simultaneously. Pathological findings of the rectus muscle and endometrial biopsies revealed endometrioid adenocarcinoma (grades 2 and 1, respectively) (shown in Figure 1). The patient had undergone C-section twice in the past. Positron emission tomography (PET)/computed tomography, performed for primary focus investigation, revealed pathologic fluorodeoxyglucose uptake in the uterine cavity and biopsy site, consistent with the findings of the residual tumor, without any pathologic uptake elsewhere (Figure 1). Gynecological examination revealed no pathological changes. A transvaginal ultrasound (US) study showed irregular thickening (up to 20 mm) of the endometrial cavity. As a result of sonographic evaluation, tumor was considered to have infiltrated less than half of the myometrium thickness. The result of CA-125 test was within the reference range. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and systematic pelvic and para-aortic lymphadenectomy, and the residual tumor in the left rectus muscle was excised. No signs of peritoneal endometriosis were observed. In addition, postoperative complications were absent, and the patient was discharged from the hospital on the 4th postoperative day. Pathological definitive diagnosis was stage-IA FIGO grade I, lymph-vascular space invasion negative, tumor invasion depth of 0.8 cm, size of 1.8 cm, and endometrioid-type endometrial adenocarcinoma; the rectus muscle excision specimen revealed grade II endometrial-type adenocarcinoma in a background of endometriosis with intact surgical margins. This case was discussed by the attending physicians at the multidisciplinary gynecologic oncology tumor board. Chemoradiotherapy was given as four cycles of carboplatin + paclitaxel combination, and radiotherapy administration was planned due to the poor malignant transformation prognosis of endometriosis in the abdominal wall. The patient was given a total of 50 Gy external-beam radiotherapy at a dose of 1.8 Gy once a day using the volumetric arc therapy method for 28 days. The patient was followed up for 3 years. At a postoperative follow-up in the 4th year, no relapsed tumor or metastasis was seen on imaging. The patient was recommended to continue follow-up at every 6 months.

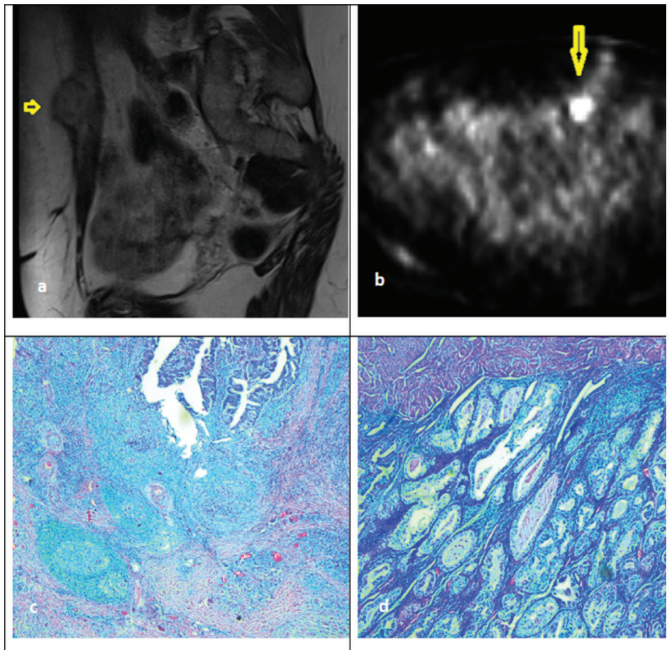

Figure 1. a) Axial section of anterior abdominal wall magnetic resonance imaging, arrow shows residual tumor nodule, b) A positron emission tomography scan showing a residual tumor on the anterior abdominal wall, arrow shows residual tumor on the anterior abdominal wall, c) Pathologic image of endometrioid grade II tumor on the anterior abdominal wall, d) Pathologic image of endometrioid endometrial grade I tumor

Discussion

This is the only case report in literature with the synchronous development of endometrioid-type endometrium cancer and endometrioid carcinoma originating from the endometriosis foci in a scar tissue. Endometriosis in a scar tissue starts with the formation of functional endometrium tissue followed by the inoculation of dropped endometrial cells into the subcutaneous or subfascial tissue inside the incision. Periodic pain and swelling in the scar tissue or surrounding nodular lesion during menstrual cycle without dysmenorrhea or pelvic pain are suggestive of scar endometriosis. It is usually located in C-section scar and inside rectus sheath until linea alba level or in subcutaneous space inside the incision scar; however, scar endometriosis in different sites such as trocar inlet, episiotomy scar, and appendectomy scar has also been reported[3]. Malignant transformation of endometriosis associated with surgical scars is extremely rare, with an estimated incidence below 0.3-1.0%, and 80% of endometriosis-related cancers are referred to the ovary[6].

Mihailovici et al.[7] analyzed data from 48 cases with endometriosis-associated abdominal wall cancer. All patients had undergone a uterine surgery, mainly C-section. The mean time between the first uterine surgery and cancer diagnosis was 19 (±8) years. The patient reported in our study had undergone C-section twice, i.e., 5 and 10 years prior to cancer diagnosis. However, the tumor progressively enlarged inside the abdominal wall within 6 months before diagnosis and caused symptoms.

Possible underlying malignancy should be considered in endometriotic foci with progressive growth. Patient's history

and results from biopsy specimen help to make a diagnosis, and US and magnetic resonance imaging studies are utilized for imaging.

In our case, the distinction between metastasis and synchronous tumor is that the grade of the lesion on the abdominal wall is higher than the cancer in the uterus, and PET scintigraphy revealed no findings in favor of metastasis in another region and pathological lymph node involvement as both imaging and pathological results were interpreted negative in favor of synchronous tumor.

Excision with intact margins should be performed in patients diagnosed with scar endometriosis or carcinoma with underlying scar endometriosis. In our case, tumor size in the abdominal wall was 5 cm, without postexcisional abdominal defect. Depending on the size of the tumor, using a mesh or flap to repair the defect in bulky tumor excisions is sometimes necessary.

The most common histological type among reported cases was clear-cell and the second most common type was endometrioid-type. It was reported that in addition to surgical treatment, 74% and 30% of cases received adjuvant chemotherapy and radiotherapy, respectively. The most frequently used adjuvant chemotherapy regimen was carboplatin-paclitaxel combination. The mean overall survival rate in 5 years was reported as 40%[7,8].

Endometriosis-associated abdominal wall cancer is a rare event, and only a few numbers of cases were reported in literature. Adjuvant therapy for these patients is not standardized[9]. It is also reported that this condition has extremely poor prognosis. Further studies and long-term results are needed to come up with an optimum treatment and approach.

Ethics

Informed Consent: Written informed consents were obtained before the study preparation.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.T., T.T., Concept: O.Ş., N.T., T.T., Design: O.Ş., N.T., T.T., Data Collection or Processing: O.Ş., N.T., Analysis or Interpretation: O.Ş., N.T., Literature Search: O.Ş., N.T., T.T., Writing: O.Ş., N.T., T.T.

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